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SOURCES OF MICROORGANISMS IN URBAN RUNOFF

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This report documents findings related to sources of microorganisms available for removal by urban runoff in well-defined residential drainage areas in Baltimore City, over an 8 month period. High densities of indicator microorganisms were found in urban sites that had high population densities and were generally in poor sanitary condition, as compared to a site that had a low population density and was well-maintained. It is highly probable that the microbial quality of runoff from "dirty" sites would improve significantly if more attention were paid to upgrading sanitation and stricter envorcement of litter laws in these areas.

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SOURCES OF MICROORGANISMS IN URBAN RUNOFF

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ABSTRACT

The sources of microorganisms available for removal by urban runoff in well-defined residential drainage areas in Baltimore City were investigated over a period of 8 months. Samples of animal feces, soil, vegetation, refuse, and dust and dirt from street surfaces were collected for microbial assay from selected representative alleyways in drainage areas of varying population densities. The alleyways were then flushed to correlate the microbiological quality of runoff to identified sources in the drainage areas.

High densities of indicator microorganisms were found in urban sites that had high population densities and were generally in poor sanitary condition, as compared to a site that had a low population density and was well-maintained. Animal feces ware found to contain high levels of indicator microorganisms and may form a large reservoir for potential microbial pollution of runoff waters. Refuse was also found to be a potential contributor of large numbers of microorganisms to runoff. It is highly probable that the microbial quality of runoff from "dirty" sites would improve significantly if more attention were paid to upgrading sanitation and stricter enforcement of litter laws in these areas.

Low levels of Salmonella were detected in animal feces, but not in runoff, soil, vegetation or street surface dust and dirt samples. The extent to which the potential load of Salmonella from animal feces could be incorporated into runoff was not clear.

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INTRODUCTION

The Jones Falls Urban Runoff Project is one of 28 projects sponsored by the U.S. Environmental Protection Agency's Nationwide Urban Runoff Program. The primary intent of the program is to obtain the information neccessary to assess the possible presence and magnitude of the water quality problems created by urban runoff.

One of the water quality problems posed by runoff from urban areas is that it has been demonstrated to contain large numbers of microorganisms that could contribute to the microbial degradation of receiving waters. When considering controls to improve the microbiological quality of receiving waters, an evaluation of the potential sources of microbial input is particularly important. The high densities of bacteria found in urban runoff may be associated with a variety of sources, such as animal fecal material, dust and dirt from streets, refuse, soil and vegetation. In addition, the microbial quality of runoff from an urban area may depend on such factors as land use, population density and the state of general sanitation in the community.

LITERATURE REVIEW

BACTERIOLOGICAL POLLUTION OF WATER - INDICATOR ORGANISMS

Total and fecal coliforms and fecal streptococci have been employed as indicators of fecal contamination of waters for many years. The coliform group is currently defined as "aerobic and facultative anaerobic, gram-negative, nonspore forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35 C"(APHA,1980). Use of this group of organisms as an indicator for potable water has been successful in controlling the major reportable contagious diseases for several decades (Pipes,1978). The fecal coliforms are a subgroup of the coliform group and are currently recognized as "coliform bacteria that ferment lactose with gas within 24 hours at 44.5 ± 0.5°C". They have been employed in the evaluation of effluents and surface waters, and are believed to be more indicative of the feces of warm-blooded animals (Geldrich,1972a), although their presence does not distinguish between human and animal fecal contamination. The fecal streptococcus group are considered synonymous with "Lancefield's Group D Streptococcus" and are defined to comprise the following species: *S.faecalis*, *S.faecalis* var *liquefaciens*, *S. faecalis* var *zymogenes*, *S. faecium*,

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S. faecium var durans, S. bovis and S. equinus (APHA, 1980). The more restrictive term "enterococcus" excludes S. bovis and S. equinus. These two organisms may be indicative of non-human animal pollution and S. bovis has been used as a specific indicator of farm animal pollution (Tilton and Litsky,1967; Oragui,1981). Fecal streptococci have been used to assess the quality of streams, lakes and estuaries. These organisms are present in large numbers in feces but are considerably less numerous than fecal coliforms in human feces.

Although no perfect indicator system has yet been discovered, the coliform group and streptococci have many desirable characteristics (Mallard,1981) and water standards are written in terms of coliforms rather than pathogens. Nevertheless, critical reviews have been made of their use (Dutka,1973a). Often, the problem lies not with the indicator systems, but with the use of bacterial indicator data in inappropriate ways, without proper regard for the limitations, to reach sweeping conclusions (Pipes,1982). Attempts to establish more reliable indicators have been and are continuing to be carried out (Carberry and Stapleford, 1970; Cohen and Shuval,1973; Levin,1977). In addition, continuing efforts are being made to refine the methods used to detect indicator organisms (Dexter,1981; Grabow et al.,1981).

RUNOFF AS A FACTOR IN BACTERIOLOGICAL POLLUTION

Runoff water from land surfaces contains a variety of bacteria, and has been recognized as a major factor contributing to the pollution of available water. The high levels of microorganisms indicative of fecal contamination in runoff waters has been well documented in the literature. High densities of total coliforms, fecal coliforms and fecal streptococci have been reported in stormwater from agricultural (Lin _et al_.,1974; Harms _et al_,1975) and rural regions (Evans _et al_.,1968; Betson and Buckingham,1969; Kunkel,1970; Doran and Linn, 1979), woodlands (Geldrich _et al_.,1968; Betson and Buckingham,1969; Davis _et al_.,1976), as well as urban and suburban drainage areas (Weibel _et al_.,1964; Burm and Vaughn,1966; Whipple _et al_., 1974; Olivieri _et al_., 1977).

The microbiological character of runoff from any given storm and any given area is likely to be unique (Mallard,1981). Among several other factors, the characteristics of the environment are likely to play a significant part in determining the quality of the runoff. For instance, urban stormwater and runoff from rural or agricultural land will very likely have different levels and nature of contaminants. Even within the narrower category of urban runoff, different types of land use and varying population densities may result in considerable differences in runoff quality. Table 1 presents a compilation of the results from several studies and gives an indication of the wide variation in levels of indicator microorganisms in stormwater runoff from different localities.

FECAL COLIFORMS/FECAL STREPTOCOCCI RATIOS

The ratio of fecal coliforms/fecal streptococci (FC/FS) has been given considerable attention with regard to the source of the polluting bacterial groups. In 1969, Geldrich and Kenner suggested that the FC/FS ratio could be used to determine whether the pollution was of human or animal origin, their data supporting the conclusion that the FC/FS ratio is greater than 4.0 in human feces and in water polluted with human waste, and less than 0.7 in animal feces, separate stormwater systems and farmland drainage. Table 2 gives a comparison of the FC/FS ratios in warm-blooded animal feces from their study. The FC/FS ratio has since been utilized frequently to differentiate between human and animal sources of pollution (Smith and Twedt,1971; Geldrich, 1972a; Feachem,1974; Skinner et al.,1974). Doran and Linn (1979) have expanded on this by using the FC/FS ratio and numbers of Streptococcus bovis in pasture

Table 1. DENSITIES OF INDICATOR BACTERIA IN STORMWATER

Reference: Mallard, 1981

Source of stormwater	Total coliforms per 100 ml.	Fecal coliforms per 100 ml.	Fecal strep per 100 ml.	FC:FS Ratio	Reference
Detroit - urban street catch basins, 1949	25,000 - 930,000	not measured	not measured	not measured	Weibel and others, 1964
Detroit - urban street catch basins, 1950	2,300 - 430,000	"	"	"	"
Seattle - street gutters 1959-1960	up to 16,100	"	"	"	"
Stockholm, Sweden - streets and parks, 1945-1948	median: 4,000 high: 200,000	"	"	"	"
Pretoria, South Africa - residential, park, school, sports ground areas	240,000	"	"	"	"
Pretoria, South Africa - business and flat area	230,000	"	"	"	"
Cincinnati, Ohio - residential; 1962-1963	median 58,000	median 10,900	median 20,500	0.53	
Cincinnati, Ohio - 1962-1964					
Wooded hillside					Geldreich and others, 1968
Spring	2,400	190	940	0.20	
Summer	79,000	1,900	27,000	0.70	
Autumn	180,000	430	13,000	0.03	
Winter	260	20	950	0.20	
Street gutters					
Spring	1,400	230	3,100	0.07	
Summer	90,000	6,400	150,000	0.04	"
Autumn	290,000	47,000	140,000	0.34	
Winter	1,600	50	2,200	0.02	
Business district					
Spring	22,000	2,500	13,000	0.19	"
Summer	172,000	13,000	51,000	0.26	
Autumn	190,000	40,000	56,000	0.71	
Winter	46,000	4,300	28,000	0.15	
Rural					
Spring	4,400	55	3,600	0.02	"
Summer	29,000	2,700	58,000	0.05	
Autumn	18,000	210	2,100	0.10	
Winter	58,000	9,000	790,000	0.01	
Baltimore - Inner city, residential commercial - Pop. density 92.5/acre, 1974-1975	mean of 24 storms 380,000	mean of 24 storms 83,000	mean of 24 storms 560,000	90% less than 4.0; 80% less than 1.0	Olivieri and others, 1977.
Baltimore - Residential/shopping center - Pop. density 26.6/acre, 1974-1975	mean of 21 storms 38,000	mean of 21 storms 6,900	mean of 21 storms 50,000	90% less than 4.0; 80% less than 1.0	"
Business district	not measured	13,000	51,000	0.26	Geldreich and Kenner, 1969
Residential	"	6,500	150,000	0.04	"
Rural	"	2,700	58,000	0.05	"
Burlington, Ontario - single-family residential	10,000	4,000	4,000	1.0	Qureshi and Dutka, 1979

Table 2. BACTERIAL DENSITIES IN WARM-BLOODED ANIMAL FECES

Reference: Geldrich *et al*., 1969

Fecal Source	Number of Samples	Densities/gram* Fecal Coliform	Densities/gram* Fecal Streptococci	Ratio FC/FS
Human	43	1.3E7	3.0E6	4.4
Animal pets				
Cat	19	7.9E6	2.7E7	0.3
Dog	24	2.3E7	9.8E8	0.02
Rodents	24	1.6E5	4.6E7	0.04
Livestock				
Cow	11	2.3E5	1.3E7	0.2
Pig	11	3.3E7	8.4E7	0.04
Sheep	10	1.6E7	3.8E7	0.4
Poultry				
Duck	8	3.3E7	5.4E7	0.6
Chicken	10	1.3E6	3.4E6	0.4
Turkey	10	2.9E5	2.8E6	0.1

* Median values

runoff to identify and evaluate the relative fecal contributions from domestic livestock and wildlife.

Some recent studies have emerged with conflicting information on the FC/FS ratio in the feces of animals and birds. Hussong et al. (1979) suggested that the FC/FS ratios of animals and birds may not be useful to separate avian from human fecal contamination since the feces from some water fowl were reported to have ratios similar to those found for human feces. The diet on which the birds were maintained seemed to be an important factor in determining the FC/FS ratio. Water fowl feeding in the wild had high ratios (3.9 and 9.2), while fasting or captive fowl had low ratios (0.1 and 0.6). Two other studies (Wheater et al.,1979; Mara and Oragui,1981) found FC/FS ratios of more than 4.0 in fecal specimens from cattle, sheep, pigs, ducks, and turkeys. In addition, ratios intermediate between 0.7 and 4.0 were often obtained for these and other animals. A portion of this data was reproduced in Table 3.

It would thus seem that FC/FS ratios must be interpreted with caution. Geldrich and Kenner (1969) also suggested that the ratios were most meaningful with respect to water quality when the microbial density data are collected near the source of contamination.

Table 3. RANGES AND ARITHMETIC MEANS OF FECAL COLIFORM TO FECAL STREPTOCOCCI RATIOS IN FECAL SAMPLES FROM VARIOUS SOURCES

Reference: Mara and Oragui, 1981

Source of Sample	Number of Samples	Range (arithmetic mean) of FC/FS ratio
Human	17	16.6-12,353 (1,778)
Cattle	8	1.2-688 (102)
Sheep	7	0.02-3,404 (524)
Pig	8	14.8-16,364 (2,562)
Dog	5	0.008-91.4 (25.9)
Rabbit	4	0.08-91.4 (25.9)
Fowl (farm reared)	4	0.3-3 (1.3)
Fowl (laboratory reared)	9	17-75 (45)
Duck	5	14-59 (31)
Seagull	5	0.04-6.4 (2.7)
Turkey	5	3.7-7.7 (4.9)

Once the microorganisms have entered the receiving body of water, numerous environmental factors and differential microbial dieaway can affect their levels. Mcfeters et al. (1974) found that the initially high FC/FS ratio of sewage decreases with time, whereas the low ratio for domestic livestock wastes increased. Geldrich and Kenner (1969) also concluded that the FC/FS ratios in stream samples would be most useful only during the initial 24 hours of downstream travel from the point of discharge. However, Olivieri et al. (1977) found that the distribution of the FC/FS ratio in urban streams was difficult to interpret even when the 24 hour limit was observed. In the case of storm drains, it was impossible to evaluate the time between the deposition of animal feces and their incorporation into the storm runoff. A large percentage of samples were in the grey area between animal (0.7) and human (4.0) contamination. The majority of raw sewage samples in this study had FC/FS ratios greater than 4.0. The FC/FS ratios in the large majority of the storm samples were less than 1.0. However, at stations representative of combined sanitary sewage and storm runoff, less than 20% of the samples had ratios greater than 4.0. In a large portion of storm samples, the presence of human contamination was masked. The FC/FS ratio should not be regarded as a "magic number" to evaluate the source of contamination in a complex system which may receive microbial contamination from a mixture of point sources as well as nonpoint sources. This observation may be especially pertinent when trying to interpret microbial data from an urban environment.

PATHOGENS IN RUNOFF

In additon to fecal indicator organisms, pathogenic bacteria have been isolated qualitatively in several instances and sometimes quantitatively from storm runoff water. Geldrich *et al.*(1968) demonstrated the existence of *Salmonella thompson* (at a level of 4500/ml) in a sample taken from an urban business district storm sewer. Claudon *et al.*(1971) isolated various *Salmonella* serotypes from a portion of Lake Mendota in Madison, Wisconsin, where one apparent source of these organisms was a residential storm sewer. Kenner and Clark (1974) also found 3 out of 6 stormwater runoff samples positive for *Salmonella*. Olivieri *et al.*(1977) investigated the occurrence of pathogens in urban stormwater in Baltimore. Table 4 gives a list of pathogens that were found in a site where no sewage overflows were known to be located. Qureshi and Dutka (1979) qualitatively detected *Salmonella* during 4 of 12 storm events at one site. *S. thompson* and *S. typhimurium* var *copenhagen* were 2 species isolated and identified in their study.

Table 4. DENSITIES OF PATHOGENS IN STORMWATER* FROM BALTIMORE, MARYLAND

Reference: Olivieri _et al_., 1977

Pathogen	Percent occurrence in samples collected	Geometric mean density
Salmonella species	52	5.7/10liters
Pseudomonas aeruginosa	100	592/100ml
Staphylococcus aureus	82	12/100ml
Animal virus	83	ND
Polio virus	42	ND
Coxsackie Virus B	50	ND
Echovirus	33	ND
other unidentified virus	8	ND
Enterovirus	ND	170 Plaque forming units

* Northwood site - no demonstrable sanitary sewer input

ND None detected

POTENTIAL HEALTH HAZARD

Although it has been demonstrated that stormwater does contain pathogenic microorganisms, generally these are present in relatively low numbers. Assessment of the potential health hazard of a particular pathogen will depend on several factors, which include the numbers of organisms ingested, the virulence of the bacterial strain and the susceptability of the human population affected (which in turn depends on such considerations as age, physiological state and degree of immunity). Olivieri et al.(1977) found that the mean Salmonella density in urban streams in Baltimore was low, with an average Most Probable Number (MPN) of 8.7/10 liters. The authors calculated that even if this water were to be consumed at the maximum possible intake of 2 liters/capita, the number of Salmonella ingested at the worst condition in the study (a maximum of 320 Salmonella/10 liters or 32/liter) would still be far below the minimal infective dose of 100,000 organisms. On the other hand, infection and resulting illness due to Shigella can be initiated with as few as 10 to 100 organisms (Bryan,1974). For enteroviruses, the minimum infective dose may be even smaller, although the exact quantity that must be ingested to produce injurious infection is not known. In general, a conservative approach is taken and one virus particle is considered sufficient to establish infection (Mallard, 1981). It should be noted however, that infection does not always result in overt disease.

Many of the studies on bacterial pathogens in runoff and surface waters approach the issue qualitatively, often dealing with frequency of isolation of the organism in question rather than its quantification in the waters examined (Claudon _et al_.,1971;Cherry _et al_., 1972; Dutka and Bell,1973b; Qureshi and Dutka,1979). It would be difficult to apply such data in assessing the health risk for an organism like _Salmonella_ whose reported ID_{50} (for healthy adults) is 10^5 to 10^7 organisms (Hornick _et al_., 1970). Enumeration of bacterial pathogens combined with knowledge of their infective doses might provide a better estimate of the direct health hazard of urban runoff. This information, along with epidemiological evidence, could give a clearer idea of the relation between bacterial disease and human contact with runoff waters. However, despite considerable advancement in recent decades, enumeration methods for most pathogens are still time-consuming, expensive and often imprecise; and there are many difficulties with epidemiological studies of waterborne disease transmitted by natural waters (Cabelli,1977).

Some controversy exists as to the actual health hazard posed by stormwater runoff. Van Donsel et al. (1967) cite a U.S. Public Health Service analysis of waterborne disease outbreaks in the United States and Puerto Rico from 1946 to 1960 which stated that "at least 29 outbreaks involving 9233 cases were associated with stormwater runoff, caused either by rainfall washing human and animal feces or sewage into wells, springs, streams, reservoirs and open water mains, or by widespread flooding of individual and public water systems". From the Baltimore study, Olivieri (1978) contends that, with the relatively low densities of pathogenic microorganisms in urban storm runoff, it is highly unlikely that urban populations will consume the large quantities of these waters required to cause overt disease, hence the enteric health hazard is limited. The commercial harvesting of shellfish from the immediate vicinity of urban storm sewers is forbidden due to unsatisfactory sanitary survey, if not for excessive coliform densities. However, some recreational taking of shellfish by individuals may present a plausible threat since pathogens are concentrated and shellfish often consumed raw. Beaches may be closed if coliform standards are exceeded when urban stormwater contaminants are washed into the recreational waters, the most common standard used for bathing waters being 200 fecal coliforms/100 ml (Brenniman et al.,1981).

SANITARY SURVEY

At this juncture, it might be appropriate to point out that qualitative and quantitative determinations of bacterial indicator density were never intended to be the only criteria for the evaluation of water quality. Along with the microbiological analysis, a sanitary survey and field observation have always been recommended, but unfortunately, are often omitted. Early workers, lacking sophisticated technology, depended heavily on the sanitary survey to evaluate the fecal contamination of water supplies (Chadwick,1842; Shattuck et al.,1917). These surveys included information on the source of the water, the topological and geological characteristics of the area, the soil characteristics and the sources of possible contamination. The importance of the sanitary survey was reiterated by a Commission appointed by the U.S. Treasury Department on Drinking Water Standards in 1914, and subsequent advisory committees on drinking water standards in 1925 and 1943, as well as the U.S. Public Health Service in the "Manual of Recommended Water Sanitation Practice" in 1944 (Olivieri,1982). While the emphasis of these reports was on drinking waters, equal weight should be given to the sanitary survey in the investigation of other waters that come into contact with humans. Information gained from field observation may be invaluable when attempting to determine the possible sources of pollution.

SOURCES OF MICROORGANISMS IN RUNOFF

RAINWATER CONTAMINATION

Rainwater itself contains insignificant levels of bacteria. Geldrich et al.(1968) collected rainwater from 49 storms from June 1965 to February 1967 and determined the levels of indicator bacteria in the samples. The median values of the bacterial counts obtained are shown in Table 5. More than 85% (42 out of 49) of these samples had counts of <1 organism per 100 ml and the remaining 7 samples had total coliform densities ranging between 1 and 92 per 100 ml. Bacteriological examination of these rainwater samples was done by membrane filtration. Microscopic examination of the filters revealed soil particles and insect as well as vegetation fragments. The bacteria were believed to have been associated with these debris.

Since rain falling to the earth contains insignificant concentrations of bacterial contaminants, the major contamination of this basic water source must then occur on contact with the polluted land environment. Betson and Buckingham (1969) demonstrated this by showing how the concentration of total and fecal coliforms during a storm varied considerably at different locations in the sampling site as shown in Table 6. Water from roofs and foundation drains had low concentrations of microorganisms while water from samples associated with overland flow had very high concentrations.

Table 5. BACTERIAL COUNTS IN RAINWATER SAMPLES

Reference: Geldrich *et al*., 1968

Season	Total coliform counts*/100ml	Fecal coliform counts*/100ml	Fecal Streptococci counts*/100ml
Spring	<1.0	<0.3	<1.0
Summer	<1.0	<0.7	<1.0
Autumn	<0.4	<0.4	<0.4
Winter	<0.8	<0.5	<0.5

* Median values

Table 6. LEVELS OF TOTAL AND FECAL COLIFORMS AT VARIOUS SITES WITHIN A DRAINAGE AREA

Reference: Betson and Buckingham, 1969

Sample site	Time	MPN/100ml Total Coliform	MPN/100ml Fecal Coliform
Gage (Q=0.3cfs)(8.51/sec)	1156	510,000	7,300
Foundation and roof drain tile	1207	4,500	<10
Street gutter sample below litter pile	1208	1,240,000	25,000
Roof downspout - no trees near house	1224	100	<10
Gage (Q=1.4cfs)(39.61/sec)	1229	460,000	27,000
Roof downspout - trees overhanging roof	1234	2,500	740
Overland flow at curb	1250	360,000	21,400
Overland flow and gutter flow	1258	470,000	58,000
Gage (Q=6.2cfs)(175.41/sec)	1303	340,000	44,000
Gage (Q=3.8cfs)(107.51/sec)	1323	360,000	26,000
Gage (Q=0.3cfs)(8.51/sec)	1749	43,000	8,400
Culvert draining street	1753	30,000	800
Roof downspout - trees overhanging	1800	<10	<10
Foundation and roof drain tile	1802	1,800	10
Street gutter near gage (West)	1806	26,000	3,000
Gage (Q=0.16cfs)(4.51/sec)	1807	58,000	18,000

SOIL CONTRIBUTION

A variety of surface materials can contaminate rainwater and are eventually incorporated into stormwater runoff. Of these sources, the soil environment might be suspect with regard to harboring microorganisms that could be washed out by rainfall. Randall (1956) isolated the fecal coliform Escherichia coli in 10 samples (10.2%) amd "other" coliforms in 65 samples (66.4%) out of a total of 98 soil samples from gardens, woodlands and grassy sites. Medrek and Litsky (1960) also found coliform bacteria in 270 out of 369 samples of soil taken from watersheds where past fecal contamination was known to be either non-existent or very remote. Total coliforms were detected in 71.8% and E. coli in 1.4% of the samples. In the same study, the occurrence of enterococci (Group D streptococci) was only 2.2% or 8 out of the total 369 soil samples examined. An earlier investigation (Suckling,1943) had also been unable to detect enterococci in virgin soils and sites not exposed to human and animal life. Mundt (1961) investigated the occurrence of enterococci in agricultural soil and obtained a 33.3% recovery or 34 out of 102 samples. It was found that the occurrence of enterococci in soil may be correlated with occurrence on the plant growing on the soil or with nearby enterococcal bearing plants. Geldrich and Kenner (1969) isolated enterococci in 63.3% of 22 agricultural soil samples.

Geldrich et al. (1962b) corroborated the earlier reports on fecal coliforms in soil by concluding from research data collected on 251 soil samples from 26 states and 3 foreign countries that soil in areas remote from human habitation was found generally not to contain this subgroup of coliforms except for insignificant levels of occasional contamination from wild animals. In contrast, soil in farm areas and cities populated by man receives varying levels of warm-blooded animal pollution. In 1968, Geldrich and others examined 843 stormwater samples from urban locations and observed that fecal pollution (indicated by fecal coliforms) accounted for an average of 8.6% of the median total coliforms present. They hypothesized that the remaining 91.4% of the total coliforms contributed to stormwater came from the soil. To test this, they compared the physiological type of indigenous non-fecal coliforms from 251 soils with similar coliforms from urban stormwater runoff and untreated surface waters. The results indicated a distribution similarity of the most common non-fecal coliform types in soil, stormwater and untreated surface water.

Cherry et al. (1975a) in an intensive ecologic survey of a Georgia stream examined environmental samples for Salmonella and Arizona. Twenty-four sand

and soil samples from stream beds and stream banks were included in this examination but neither Salmonella nor Arizona were detected in any of these samples. A similar study was carried out by Thomason et al. (1975) who found 8 out of 58 soil samples culturally positive for Salmonella. The sampling site was a mountain top that was the central attraction in a state park. The moisture content of the sample appeared to have a significant effect on the detection of Salmonella. All 8 positive samples comprised wet soil collected in or near standing or running water, and Salmonella could not be cultured from any of the dry samples.

The persistence and behavior of enteric bacteria in soil following contamination is also of significance in the evaluation of the microbial contribution from the soil. Mallman and Litsky (1951) compared the survival of Streptococcus faecalis and E. coli in a number of soils contained in metal cylinders. The results reported were based on 100 grams of dried soil. At temperatures of 25-28°C, S. faecalis persisted for about 40 days, whereas E. coli were still recovered after 77 days. Salmonella typhi survived for a maximum of 18 days. Salmonella have also been shown to persist in naturally contaminated soil. In one case, S. typhimurium was found in garden soil exposed to ordinary weather conditions at least 250 days after contamination, and was thought to have been either the cause or result of a human outbreak (Steele,1968). In their review of the literature, Zibilske and Weaver 1978) mention survival periods for Salmonella in manure-treated soils ranging from a few days to 2 years.

In general, survival of enteric organisms in soil has been found to depend upon environmental characteristics and soil characteristics (Rudolfs et al., 1950; Zibilske and Weaver,1978). Van Donsel et al. (1967) studied the survival of E. coli and S. faecalis inoculated into exposed outdoor plots. They noted a significant seasonal variation in survival. During summer, the fecal coliforms survived slightly longer (3.3 days) than the fecal streptococci (2.7 days); during autumn survival was the same (13 days); and in spring and winter the fecal streptococci survived longer than the fecal coliforms, persisting for as long as 20 days. Both organisms were isolated from storm-water runoff collected below a sampling site when counts were sufficiently high in the soil. Temperature conditions at the sites were observed to have an effect on the numbers of soil coliforms, with very warm weather following a rain shower causing a considerable increase. Lack of soil moisture, on the other hand, was not conducive to survival. A study on the survival and movement of fecal indicator bacteria in soil (Hagedorn et al.,1978) found that bacterial numbers peaked in sampling wells associated with the major rainfall patterns. Burge and Parr (1980), in an extensive review of papers on the movement of pathogenic organisms from waste applied to agricultural lands, also concluded that runoff from land appears to be a much more immediate and widespread mode of transport of organisms than movement through the soil profile.

CONTRIBUTION FROM VEGETATION

The occurrence of fecal indicator organisms on vegetation is of some concern as these microorganisms may be introduced into soil and water and enter ~~surface~~ waters by way of stormwater drainage. Wilson et al. (1935) found very few coliforms on grass, hay and straw except on samples that had been contaminated by soil. However, Thomas and McQuillin (1952) found these organisms abundant in samples from both ungrazed grassland and intensively grazed pasture. Fraser et al. (1956) reported that coliforms were seldom detected on foliage of a wide variety of vegetation, and speculated that exceptions were probably the result of contamination from insects, animals or dust.

Mundt et al. (1958) reported a 58.5% recovery of enterococci from 62 of 106 leaf, shoot and flower samples involving 63 plant species from agricultural sites, and coliforms were isolated from 67.0% of these samples. In subsequent studies, Mundt and various co-workers obtained enterococci from 34, 32.2 and 10.4% respectively of the flowers from nonagricultural, agricultural and grass plants (1961); and 14.2% and 3.4% enterococcus recovery respectively from 2169 flowers and 440 leaf, bud, shoot, fruit and seed samples from a variety of species in a wild environment (1963). Geldrich et al. (1964) found higher numbers of fecal streptococci than fecal coliforms (the occurrence of both was very low) on a variety of vegetation (Table 7).

Pathogens have been isolated in a few instances from contaminated vegetation. Cherry et al. (1975) isolated 3 different serotypes of Salmonella from 4 out of 8 samples of mosses, grass and weeds from an urban stream. Thomason et al. (1975) detected Salmonella in 5 out of 26 vegetation samples (19.2%) from 2 sites. As with the soil samples, all 5 vegetation samples positive for Salmonella were wet samples and the pathogen was not detected in any of the dry samples.

Table 7. MEDIAN MPN VALUES PER GRAM FOR 152 VEGETATION AND 40 INSECT SAMPLES

Reference: Geldrich *et al*., 1964

Sample Classification	Number of samples	Fecal Coliform	Fecal Streptococci
Vegetation			
Flowering heads, buds, blossoms	81	<2.0	27
Foliage of:			
Garden vegetables	27	<2.0	35
Ornamentals	15	<2.0	49
Wild flowers	11	<0.2	33
Spaghum-type peat moss	8	<4	<4
Indoor-cultivated foliage	10	<0.2	0.2
Insects			
Coleoptera (beetles)	16	<20	1,024,000,000
Orthoptera (grass-hoppers, etc.)	11	<20	790,000
Hymenoptera (bees, wasps and ants)	7	<20	49,000
Diptera-Homoptera (flies-leafhoppers)	3	<40	344,000
Lepidoptera (moths and butterflies)	3	<20	1,720

Bell (1976) reported that fecal coliforms were removed from the leaves of alfafa plants after 2 sunny days. Chandler et al. (1981) found the persistence of indicator organisms on pasture to be variable but generally less than that in soil. Some reported values of survival times of indicator organisms and infectious agents on vegetation and soils are summarized in Table 8 (Uiga and Crites, 1980).

Several possibilities have been discussed as to the origin of indicator bacteria isolated from plants (Clausen, 1977). The first is that they may be present as a result of direct contamination from wild animals and insects, or both. Mundt (1963) could find no evidence of plant-specific species of enterococci. On the basis of similarity between species recovered from species recovered from plants and animal feces, he concluded that the plants were contaminated by wild animals. Geldrich et al. (1964) observed that where there was little warm-blooded animal pollution, contamination by insects may account for some of the streptococcal population on plants, and noted that density levels of fecal coliforms and streptococci on insects were repeated in a similar pattern but at lower density levels on vegetation (Table 7). Eaves et al. (1960) had earlier demonstrated that insects could

Table 8. REPORTED SURVIVAL TIMES OF INDICATOR ORGANISMS AND INFECTIOUS AGENTS ON SOILS AND VEGETATION

Reference: Uiga and Crites, 1980

Constituent	Type of Soil or Vegetation	Survival Time (days)	Estimated Die-off After 7 Days, %*
Indicator organisms			
Coliforms	Fodder	6-34	90
	Vegetables	35	90
	Soil surface	38	88
Infectious agents			
Shigella sp.	Fodder	<2	Below detection
	Leaf vegetables	2-7	Below detection
	Orchard crops	6	Below detection
Salmonella sp.	Fodder	12-<42	94
	Soil surface	15-46	93
	Leaf vegetables	1-40	98
	Orchard crops	0.75-<2	Below detection
Enterovirus	Leaf vegetables	15-60	89
Entamoeba histolytica	Leaf vegetables	2	Below detection

* Calculated from median survival time

transfer streptococci in the environment to plants. The second explanation for the presence of indicator organisms on plants is that these bacteria may be temporary residents on plants, capable of limited reproduction, and work done by Mundt (1961) supported this theory. Simultaneous occurrence or absence of these organisms on plants and in the surrounding soil suggested that counts in the soil result from dissemination by insects, rain, wind and gravity. Another possibility is that indicator bacteria may also exist on plants in a truly epiphytic relationship. Mundt et al. (1962) also demonstrated evidence of this when they observed S. faecalis var liquefaciens to establish commensal growth on 4 types of vegetables. In addition, Geldrich et al. (1964) detected starch hydrolysis in 37.7% of 646 enterococcal cultures isolated from plants. This characteristic was uncommon to strains recovered from water, animals, insects and soil. Recently, Mundt (1975) also reported large numbers of enterococci isolated from vegetation to be physiologically different from recognized species common to warm-blooded animals.

CONTRIBUTION FROM WARM-BLOODED ANIMALS

When animals defecate directly onto the land, fecal contamination of stormwater runoff and surface waters occurs when rain falling to the ground washes the fecal wastes into receiving waters. In a rural environment, fecal pollution may be derived from livestock and poultry, with contributions in lesser amounts from wild animals and birds, while in urban areas fecal contamination probably originates from dogs, cats, rodents and other small animals (Geldrich *et al*. 1968).

The amount of waste that animals are capable of generating should not be underestimated. It has been calculated that over 1 billion tons of fecal wastes are produced by domestic animals in one year, and that as much as 50% of this waste production comes from feedlot operations (Wadleigh,1968). New York City's Environmental Protection Administration has estimated that 5000 to 20,000 tons of feces (dry weight) and 600,500 to 1,000,000 gallons of urine are excreted yearly by its 500,000 dogs (Beck,1972).

As can be seen from Table 2 (p.8), which lists median bacterial densities for some domestic pets, livestock and poultry, animals can contribute large quantities of indicator bacteria to runoff. Fecal coliforms have been found to have an excellent positive correlation with warm-blooded animal fecal contamination, forming a large percentage of the total coliform population (Table 9). Human feces contain a greater number of coliforms than streptococci. The ratio of enterococci to other streptococci occurring in feces varies among different vertebrate species. Human feces are characterized by a great predominance of enterococci (Clausen _et al_.,1977), while enterococci in animal feces has been reported to range from 10% of the fecal streptococcal population in pigs to 12.3% in cattle, 24.8% in sheep and 61.8% in fowl (Kenner,1960). Feces from livestock are known to contain significant proportions of _S. bovis_ and _S. equinus_, which are rarely isolated from human stools (Kenner,1978).

Human pathogens are also found to frequent the intestinal tracts of warm-blooded animals. These pathogenic microorganisms are potentially transmissible to humans by the contamination of water with the fecal or urinary wastes of wild and domestic animals. They include _Giardia lamblia_, _Salmonella_ spp., enteropathogenic _E. coli_, _Pasteurella tularensis_, _Leptospira_ spp., and some of the intestinal parasites (Summers,1967; Pipes,1978). Agricultural animals are frequent sources of _Salmonella_. For example, cattle feedlots, livestock salesyards and abattoirs can be major sources of _Salmonella_ that enter streams and lakes used for recreational purposes (Geldrich,1972b).

Table 9. FECAL COLIFORM TEST CORRELATION WITH ANIMAL FECES

Reference: Geldrich, 1970

Fecal Source	Fecal coliform percentage
Human	96.4
Livestock	98.7
Poultry	93.0
Cats, Dogs and Rodents	95.3

In an urban environment, dogs, cats, rodents and and other small animals may constitute a significant source of fecal pollution in runoff waters. Information that may be useful in assessing the possible health hazard from such contamination includes the occurrence of enteric pathogens in these animals. Concern about the possible transmission of disease from animals that live in intimate association with man has prompted several studies in different areas of the country on their potential for harboring pathogenic organisms. Wolff et al. (1948) isolated Salmonella types from the stools of 18 out of 100 dogs (18%) examined in Michigan. In Texas, Watt and DeCapito (1950) isolated Salmonella from 39 out of 1156 specimens (3.4%) from pet dogs and 19 out 625 samples (3.0%) from cats. Mackel et al. (1952) found 15.1% of dogs sampled in Florida positive for Salmonella and in the same state, Galton et al. (1952) detected 27.6% positives. In Georgia, Stewart and DeCapito (1953) obtained 5.1% and 1.6% positives for Salmonella in dogs and cats respectively. In both the Texas and Georgia studies, cultures were obtained from several animal species. Considering those animals cultured in significant numbers (>180), dogs had the highest prevalence rate of Salmonella, followed by ducks, cats and chickens.

Another point of similarity in the 2 studies was that the same types of Salmonella organisms were isolated from both man and animals within the same locality. Butler, who along with other investigators, carried out 2 studies (1965, 1967) on Salmonella occurrence in family pet dogs in Alaska, reported 27% positives in the first instance and 34% in the second. In the latter study, housepets were compared to other dogs on a military base and a private kennel. No positive cultures were obtained from the dogs on the base and only one from the kennel dogs. A consideration of the factors that constitute the environments of the 3 groups of dogs indicated that the acquisition and dissemination of these pathogens was related directly to the animals' freedom of movement and particularly to their access to garbage. Family pets were given much more freedom to wander and were often found using garbage containers in the city as feeding stations. In addition, there is usually an intimacy of contact between dogs and human beings, and especially so between housepets and children. This close interrelationship increases the possibility of the transmission of pathogens like Salmonella between humans and animals.

GENERAL SANITATION AND LITTER

Man's neglect of his environment may also contribute microorganisms to runoff. Olivieri (1978) has suggested that the poor sanitary conditions and sometimes massive accumulations of refuse in streets and alleys in densely populated areas may be an often overlooked source of bacteria. Betson and Buckingham (1969) reported more than a million total coliforms and 25,000 fecal coliforms/100 ml in a runoff sample collected below a litter pile.

SUMMARY

It has been demonstrated that stormwater runoff can contain high densities of indicator bacteria. Indicator ratios, particularly those of fecal coliforms to fecal streptococci, have been employed in many instances to determine if the source of bacterial pollution in runoff waters is of human or animal origin. However, some controversy exists as to the use of the FC/FS ratio. Although pathogens have been detected in several stormwater studies, a large number of these determinations were made qualitatively. There is no real agreement as to the actual health hazard that exists from storm runoff.

Rainwater is contaminated when it flows over the polluted land environment. A variety of sources may contribute microorganisms to runoff waters. Animal feces has been shown to contain high levels of indicator bacteria. These organisms have also been detected in soil and vegetation under various conditions. Refuse piles and street litter may be a potential source of large numbers of microorganisms, especially in densely populated urban areas in poor sanitary condition, but at present there is insufficient data to support this theory.

Relatively little work has been carried out to relate the levels of microorganisms in potential sources from a particular drainage area to the microbial quality of runoff from that area. A major objective of this study was to obtain such information, in the hope of gaining some additional insight as to the major contributing factors in the microbial degradation of runoff waters. Field observations of environmental conditions and land-use characteristics played a significant role in the attempt to identify major sources.

STUDY SITES - GENERAL LOCATION

The Baltimore metropolitan area, as shown in Figure 1, is intersected by 4 separate drainage areas which discharge into the Patapsco sub-estuary of the Chesapeake Bay. The principal drainage basins are the Patapsco River, the Gwynns Falls, the Jones Falls, and Herring Run. The investigations for this study were mainly carried out in 3 small homogenous catchments located within the Jones Falls watershed. These were the Homeland, Bolton Hill and Reservoir Hill drainage areas and are depicted in Figure 2. The characteristics that were used to define each small homogenous catchment are summarized in Table 10. The Homeland catchment is a fully residential community, covering an area of 28 acres with a low population density of 7 persons/acre. The Bolton Hill catchment is mainly a residential area, with some recreational land, and covers 14 acres. The population density for this catchment is moderately high, with 28 persons/acre. The Reservoir Hill catchment is also a fully residential neighborhood of about 10 acres in area, and has a high population density of 74 persons/acre.

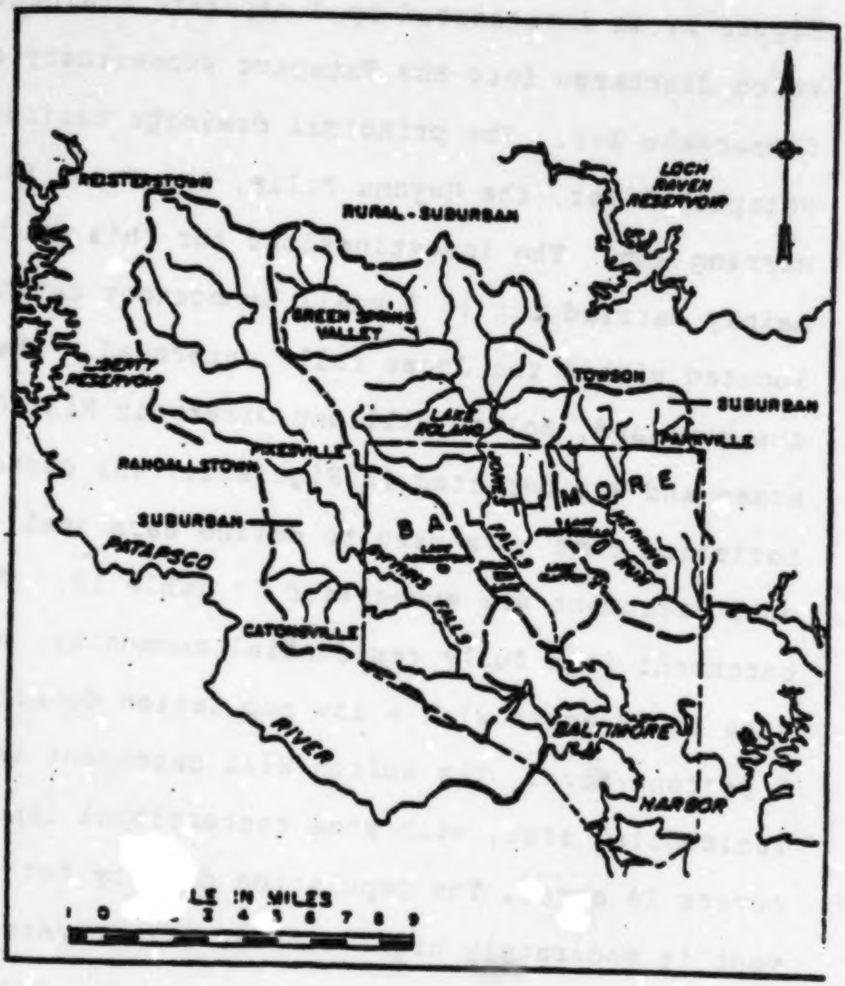
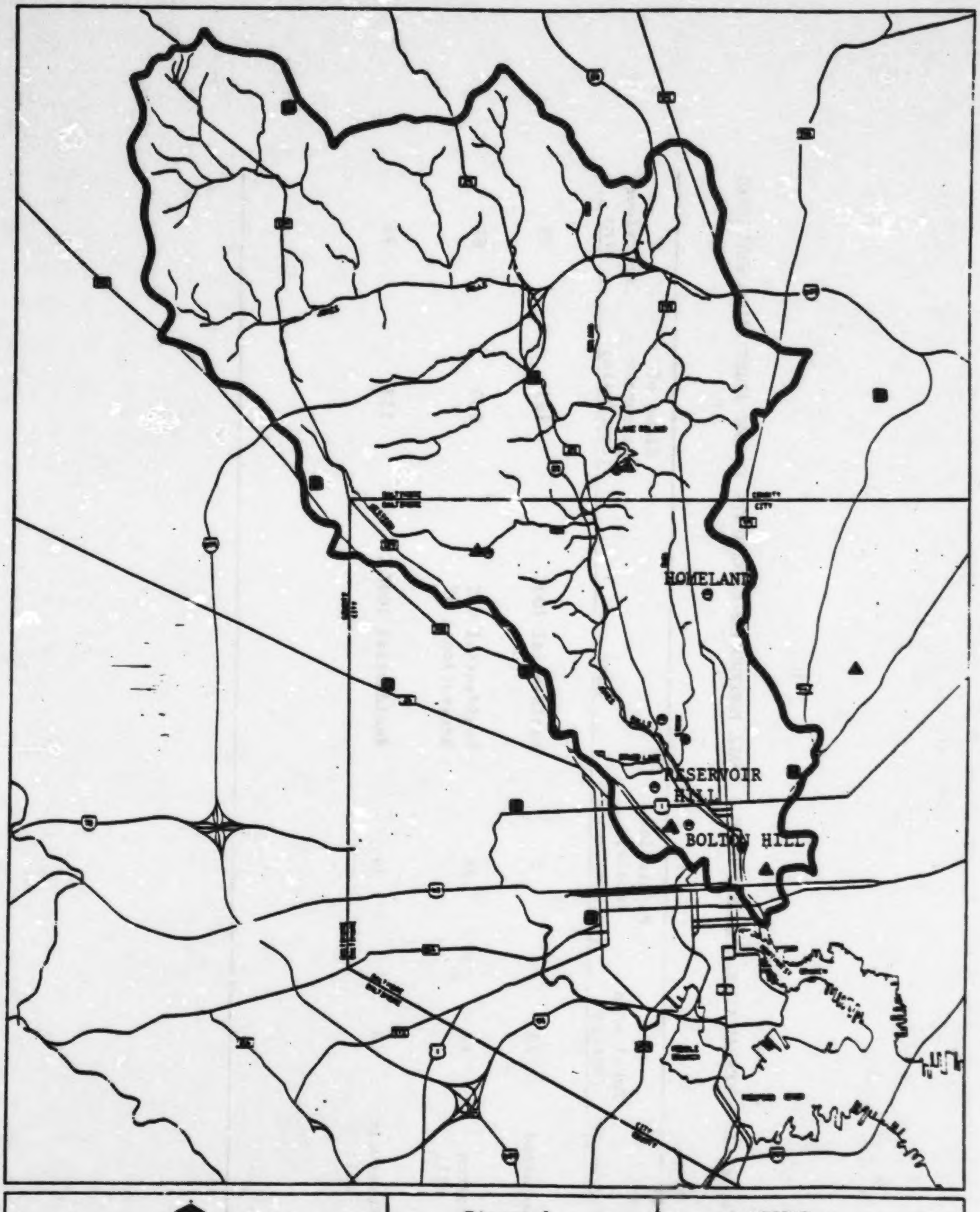

REISTERSTOWN
RURAL - SUBURBAN
LOCH RAVEN RESERVOIR
GREEN SPRING VALLEY
LIBERTY RESERVOIR
TOWSON
SUBURBAN
LAKE ROLAND
PARKVILLE
PIKESVILLE
RANDALLSTOWN
SUBURBAN
PATAPSCO
BA MORE
JONES FALLS
HERRING RUN
GWYNNS FALLS
CATONSVILLE
RIVER
BALTIMORE
HARBOR
SCALE IN MILES
1 0 1 2 3 4 5 6 7 8 9

Figure 1. The four major drainage areas of the Baltimore metropolitan area; Patapsco River, Gwynns Falls, Jones Falls and Herring Run.


HOMELAND
RESERVOIR HILL
BOLTON HILL

JONES FALLS URBAN RUNOFF PROJECT

REGIONAL PLANNING COUNCIL
BALTIMORE MARYLAND

Figure 2.

JONES FALLS WATERSHED

Location of Small Homogeneous Catchments

-LEGEND-

- ● RECEIVING WATERS STATION
- ⊖ SMALL HOMOGENEOUS CATCHMENT
- ▲ AIR DEPOSITION STATION
- ■ SUPPLEMENTAL RAINGAGE

Table 10. GENERAL CHARACTERISTICS OF 3 SMALL HOMOGENOUS CATCHMENTS IN THE JONES FALLS WATERSHED

Catchment	Total area (acres)	Population density (persons/acre)	Land use	Slope of Catchment (feet/mile)	% Impervious area
Homeland	28	7	Residential 100%	183	29
Bolton Hill	14	28	Residential 95% Recreational 5%	130	61
Reservoir Hill	10	74	Residential 100%	153	76

STUDY SITES - LOCATION WITHIN SMALL WATERSHEDS

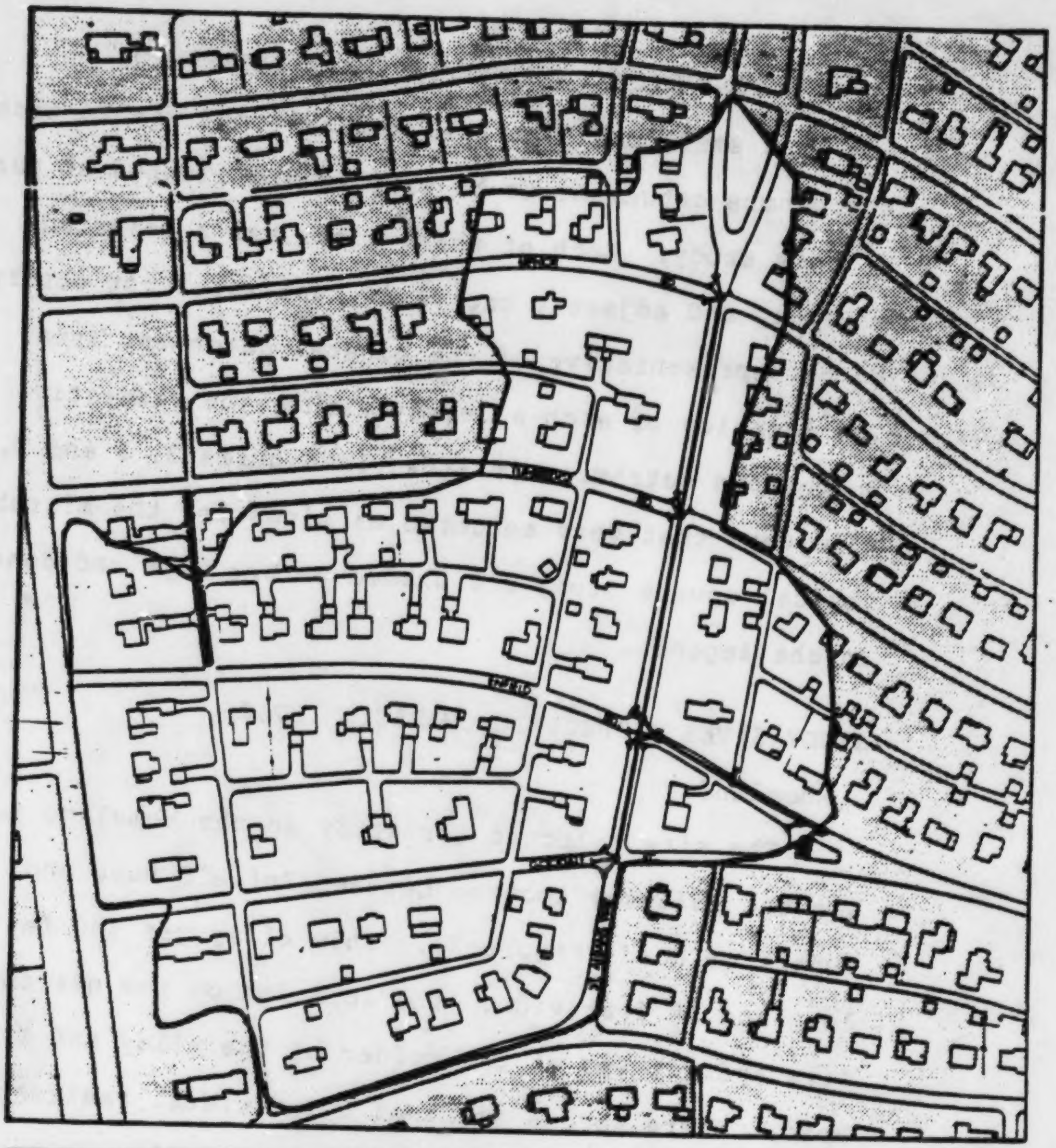
One small drainage area was selected within each homogenous catchment to be sampled and flushed as part of this study. Each of these sites was a selected alleyway and adjacent backyards, and was first observed to be representative of the general catchment area. The location of each alleyway within the respective homogenous catchment is shown in Figures 3, 5 and 7. Alleyways that were selected as sites for the microbiological source study are shaded on the maps and denoted in the legends.

STUDY SITES - SPECIFIC CHARACTERISTICS

Homeland --

The site selected for study in the Homeland area was the alleyway located between Enfield Road and Tunbridge Road (Figure 3). This alley was 350 feet long and 15 feet wide. The location of the nearest fire hydrant was at the corner of the alley and Enfield Road. There were a total of 5 individual residential buildings bordering both sides of the alley.

This study site was observed to be very well-maintained, which was not surprising since it was located in a higher income residential area with few residents. The alleyway was generally very clean and



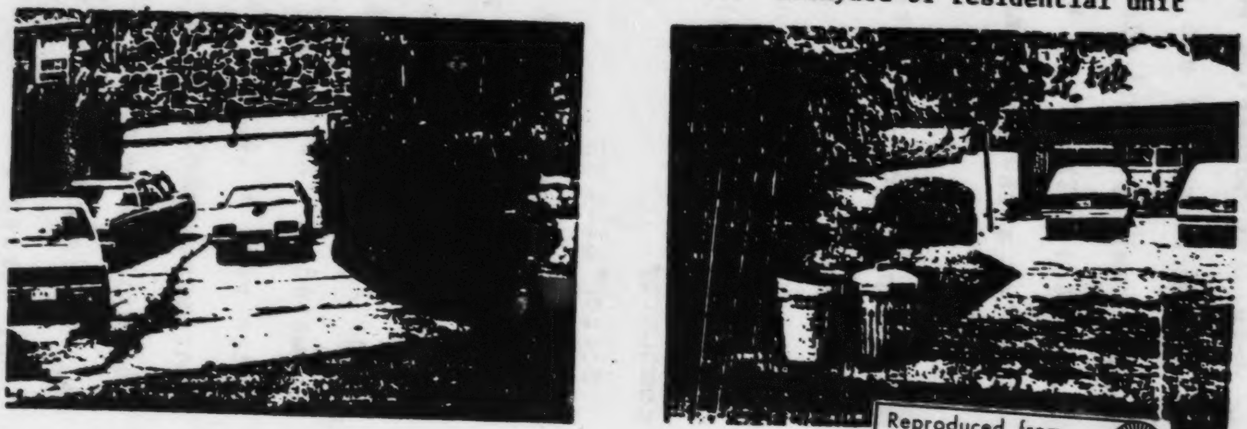
JONES FALLS URBAN RUNOFF PROJECT
REGIONAL PLANNING COUNCIL
BALTIMORE MARYLAND

Figure 3.
SMALL HOMOGENEOUS CATCHMENT
HOMELAND
28.06 ACRES

-LEGEND-
▬ MONITORING STATION
Y STORM SEWERS
▬ MICROBIOLOGICAL SOURCE STUDY

4a. View of alleyway

4b. Backyard of residential unit



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4c. Driveway of residential unit

4d. Covered trash cans

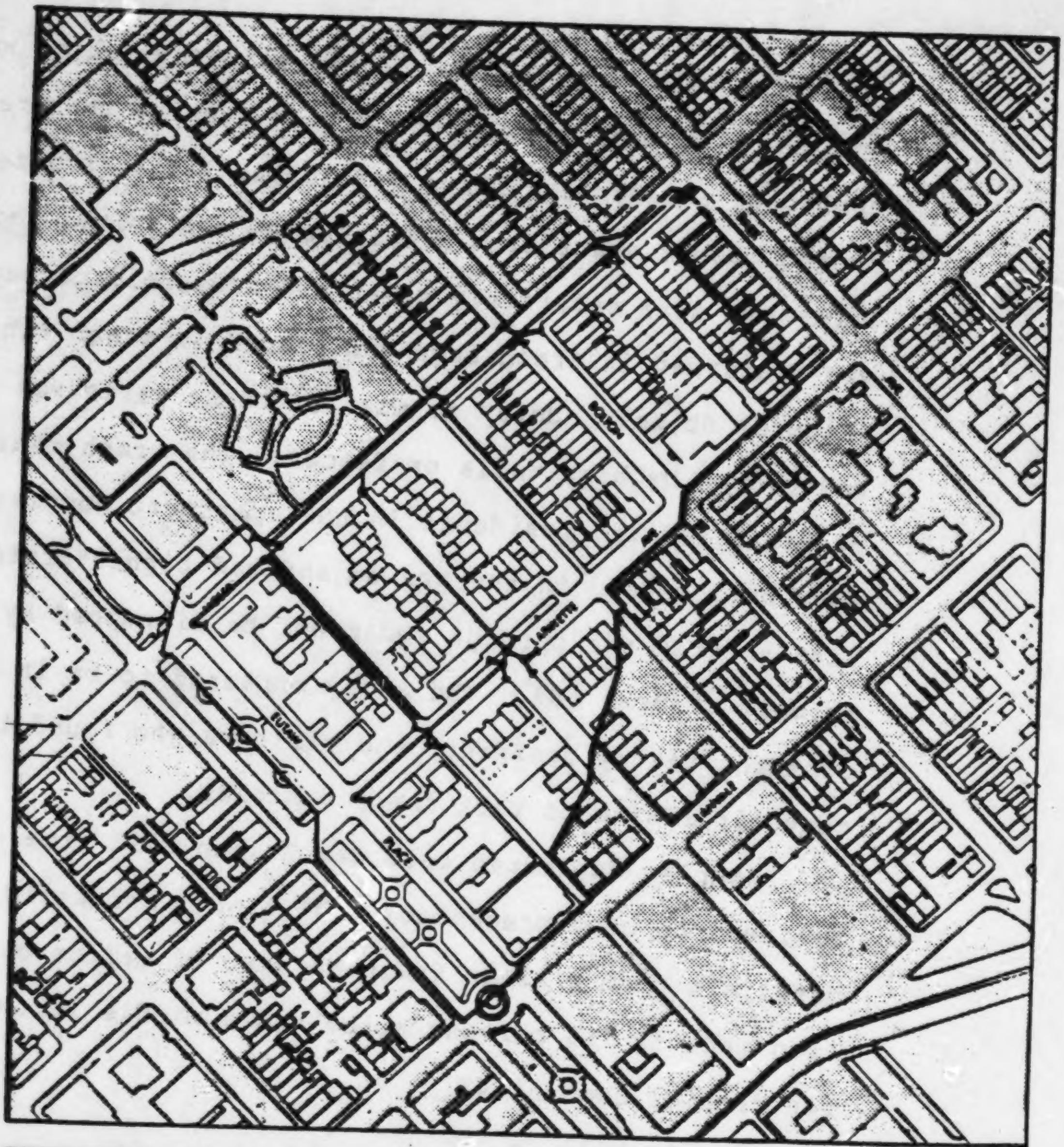
Figure 4. Characteristics of the Homeland study site.

free of litter as can be seen in Figure 4a. Backyards and driveways bordering the alley were kept clean and tidy (Figures 4b,4c) and household garbage was enclosed within trash bags and placed in covered trash cans (Figure 4d). The alley was seldom used by non-residents except for a few passers-by and people walking their dogs.

Samples collected from this site were believed to be representative of runoff and solid materials from a clean, well-kept drainage area with a low population density (7 persons/acre) and a higher income residential community.

Bolton Hill --

The selected study site in this catchment was the alleyway called Jordan Alley, located at right angles to Lafayette Ave. (Figure 5). This alley was 350 feet long, 20 feet wide, and the nearest fire hydrant was situated at the corner of the alley and Lafayette Ave. There were a total of 9 residential buildings and one apartment complex with 11 two-storey apartments bordering this alley. Located in the middle of the alley across from the apartment complex was an "island" where trash cans for the apartments were placed and some trees and ground vegetation grew.


BOLTON
LAFAYETTE
EUTAW
PLACE
LANVALE

JONES FALLS URBAN RUNOFF PROJECT
REGIONAL PLANNING COUNCIL
BALTIMORE MARYLAND

Figure 5.
SMALL HOMOGENEOUS CATCHMENT
BOLTON HILL
14.15 ACRES

-LEGEND-
▬ MONITORING STATION
Y STORM SEWERS
▬ MICROBIOLOGICAL SOURCE STUDY

Figure 6a shows a view of the alleyway, which was generally in good sanitary condition despite the greater number of residents. There was little litter scattered on the ground apart from dried leaves and animal feces. Backyards were relatively clean and household garbage was discarded in trash bags and placed in trash cans (Figures 6b,6c). Entry to most of these yards was restricted by high walls or fences. The trash cans provided for the residents in the apartment complex were half buried in the ground and had tight-fitting lids so that the garbage could not be disrupted by animals (Figure 6d). The alley appeared to be in constant use by residents and visitors who found it a convenient site to park their vehicles.

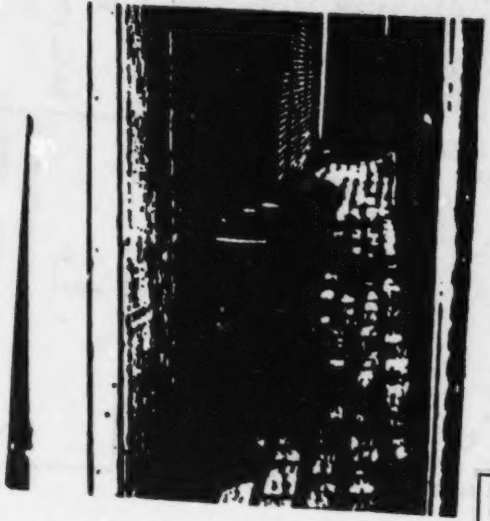
Samples collected from the Bolton Hill site were believed to be representative of runoff and solid materials from a clean drainage area of medium population density (28 persons/acre) and a middle income residential community.

Reservoir Hill --

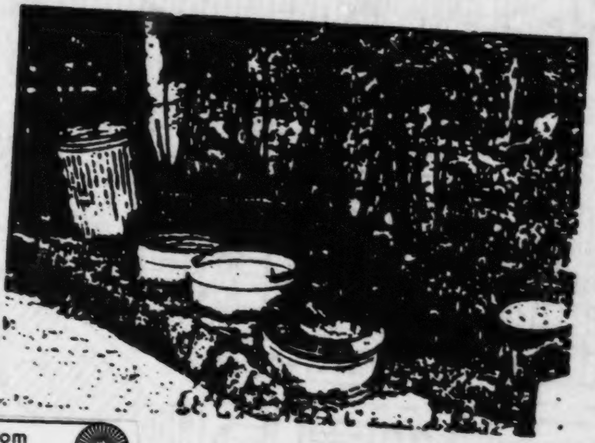
The alleyway selected for study was located between Newington Ave. and Whitelock Street (Figure 7). The nearest fire hydrant was about 150 feet away at the corner of Whitelock Street and Callow Ave. The alley

6a. View of alleyway

6b. Backyard of residential unit



6c. Backyard of residential unit



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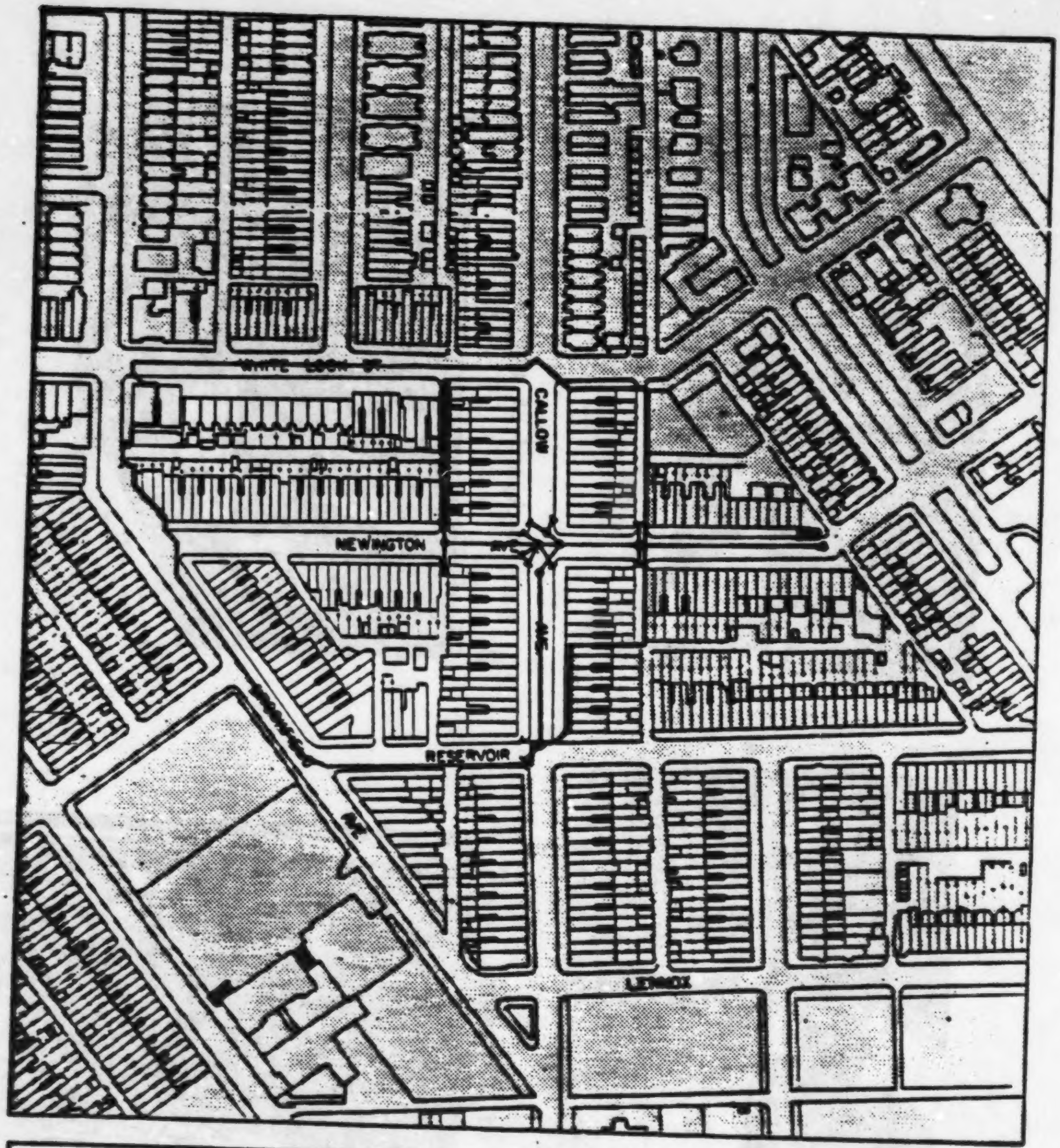
6d. Refuse disposal - trash cans

Figure 6. Characteristics of the Bolton Hill study site.


WHITE LOCK ST.
CALLOW
AVE.
NEWINGTON
AVE.
RESERVOIR
AVE.
LENNOX

JONES FALLS URBAN RUNOFF PROJECT
REGIONAL PLANNING COUNCIL
BALTIMORE MARYLAND

Figure 7.
SMALL HOMOGENEOUS CATCHMENT
RESERVOIR HILL
10.48 ACRES

-LEGEND-
▬ MONITORING STATION
Y STORM SEWERS
▬ MICROBIOLOGICAL SOURCE STUDY

was 250 feet long and 18 feet wide and was bordered by 14 rowhouses.

This site was observed to be generally in poor sanitary condition (Figure 8a). One end of the alley in particular appeared to be an unofficially designated spot for a massive pile of garbage even though there were no trash cans on this corner (Figure 8b). Some of this refuse was tied up in plastic trash bags but this did not prevent large animals like dogs from dislodging and scattering the garbage. As a result, uncovered refuse was abundant in the alleyway and a considerable amount found its way into the stormwater inlet at the lower end of the alley. The backyards adjacent to the alley were generally poorly-kept, and except in a few cases, fences that would have restricted entry were in disrepair or had been removed. There were large amounts of litter scattered in some of these backyards (Figure 8c), and even where trash cans were available, these were often without lids and trash was often observed to overflow on to the ground (Figure 8d).

Runoff and solid samples collected from this site were believed to represent input from a poorly maintained drainage area with a high population density (74 persons/acre) and a low socio-economic residential community.

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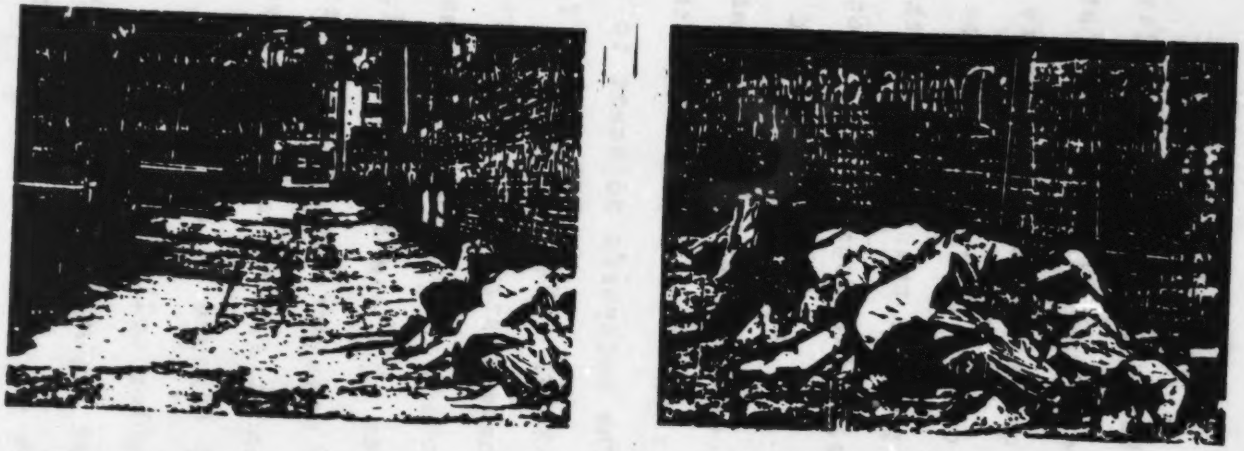
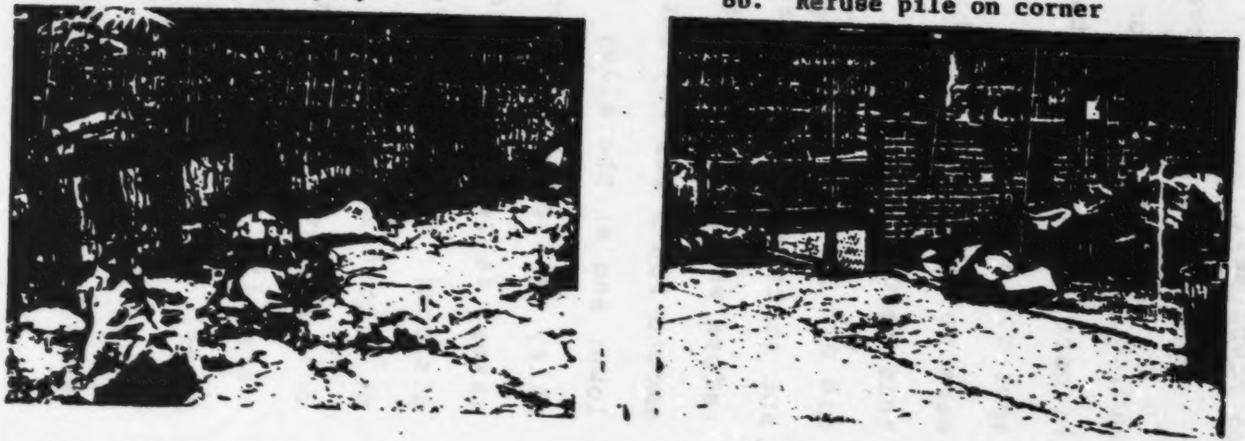


8a. View of alleyway

8b. Refuse pile on corner



8c. Refuse in backyard

8d. Overflowing trash cans

Figure 8. Characteristics of the Reservoir Hill study site.

PRELIMINARY INVESTIGATIONS

It was decided that a few preliminary trials with the alley flush system would be useful before venturing out into the 3 study sites, which were all located some distance away from the laboratory. An alleyway situated in the immediate vicinity of the Johns Hopkins School of Hygiene and Public Health was selected for for this purpose. The main reason for choosing this alley was its easy accessibility from the laboratory.

This alley in East Baltimore was 160 feet long and about 10 feet wide. The nearest fire hydrant was on a street corner about 240 feet away. One side of the alley was bordered by the backyards of 10 rowhouses, and the other side by the walls of 2 corner rowhouses.

This alleyway was similar to the Reservoir Hill site in that it was usually in an unsanitary state, with a massive garbage heap at one end (Figure 9a), and accumulations of refuse along the alley (Figure 9b), as well as occasionally in some backyards (Figure 9c). Samples collected from this site were believed to be representative of runoff and solids from an inner city residential area with a high population density and poor sanitary conditions.

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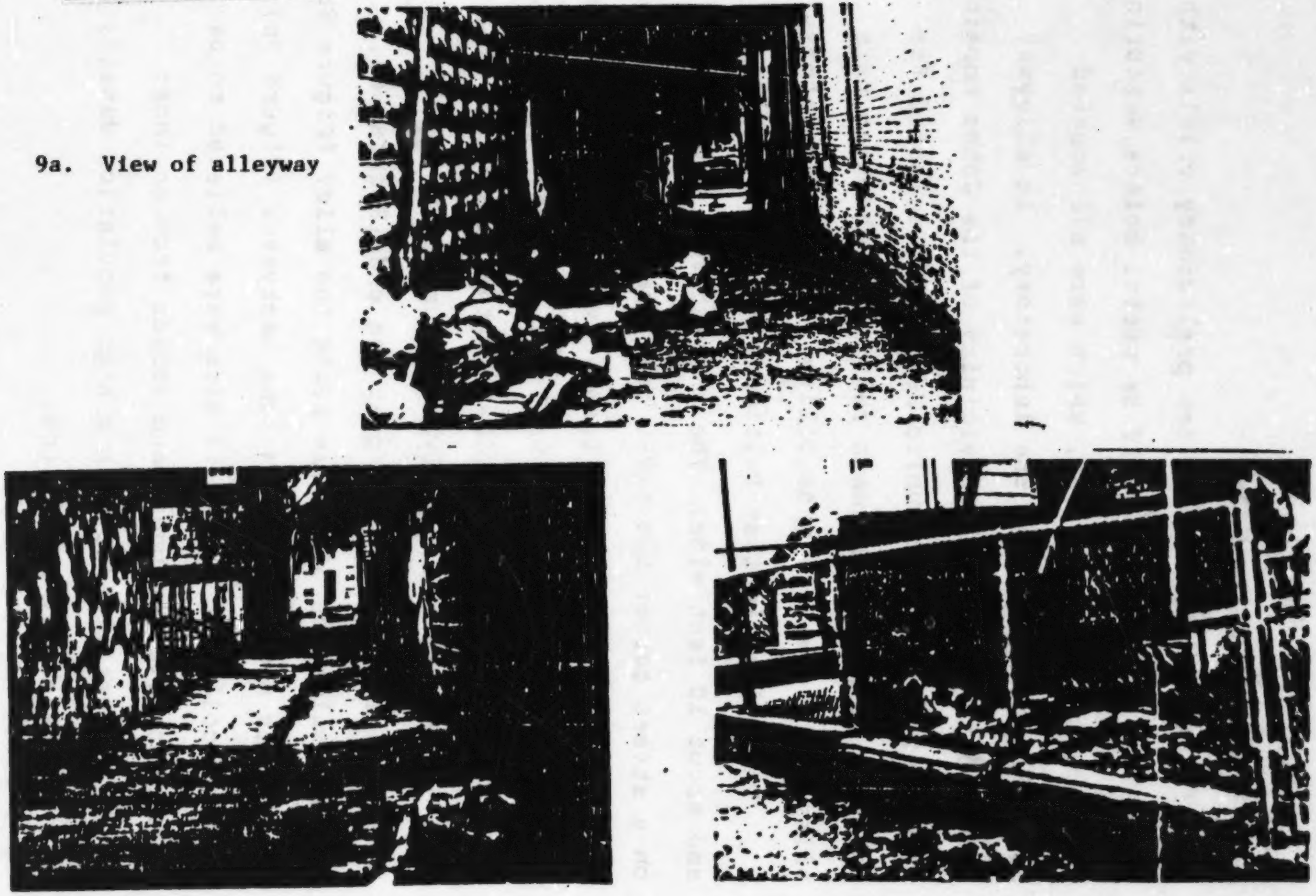


9a. View of alleyway

9b. Refuse in alleyway

9c. Refuse in backyard

Figure 9. Characteristics of the East Baltimore site.

MATERIALS AND METHODS

ALLEY FLUSH

The alley flush was conducted to simulate natural rainfall. The equipment arrangements for flushing the selected alleyways included a connection from the nearest fire hydrant to a plastic pipe (polyvinylchloride 40, 2" diameter). This pipe supplied water to a sprinkler system consisting of a series of lawn-sprinkler heads connected by the pipe down the alley. Figure 10 shows a plan view of the system and Figures 11a, 11b and 11c show the system assembled and in operation at 3 study sites. The sprinkler heads were spaced 30 feet apart along the entire length of the alley. The objective was to simulate a rainfall event over an area that would cover a width of about 60 feet, and the length of the alley for a duration of about 1.5 hours. The area affected would thus include the entire alleyway as well as backyards adjacent to the alley.

Each sprinkler unit consisted of a Sears and Roebuck Company oscillating lawn sprinkler (Catalogue # M79041) connected by 6 feet of plastic pipe (polyvinylchloride 40, .75" diameter), and a gate valve to a 2.5" reducing tee as can be seen in the following figures. Wooden stakes were used to hold the sprinkler heads up

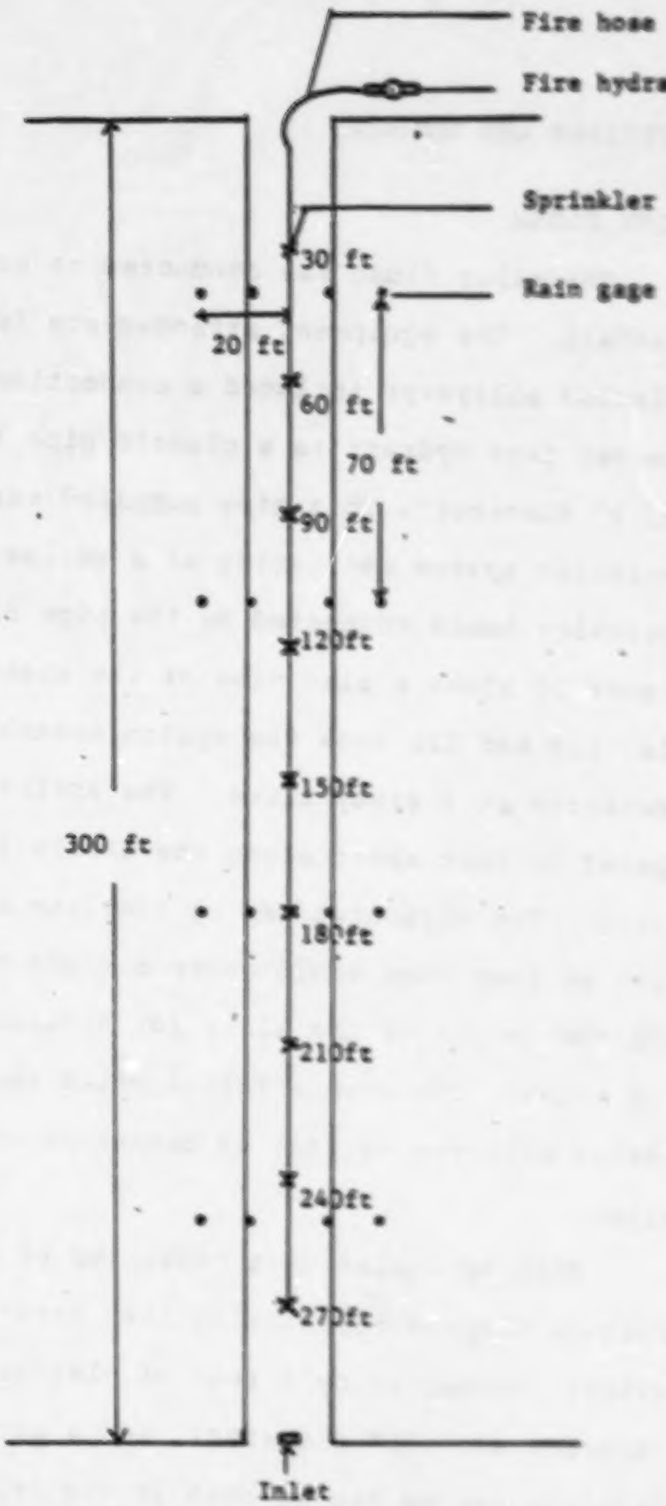
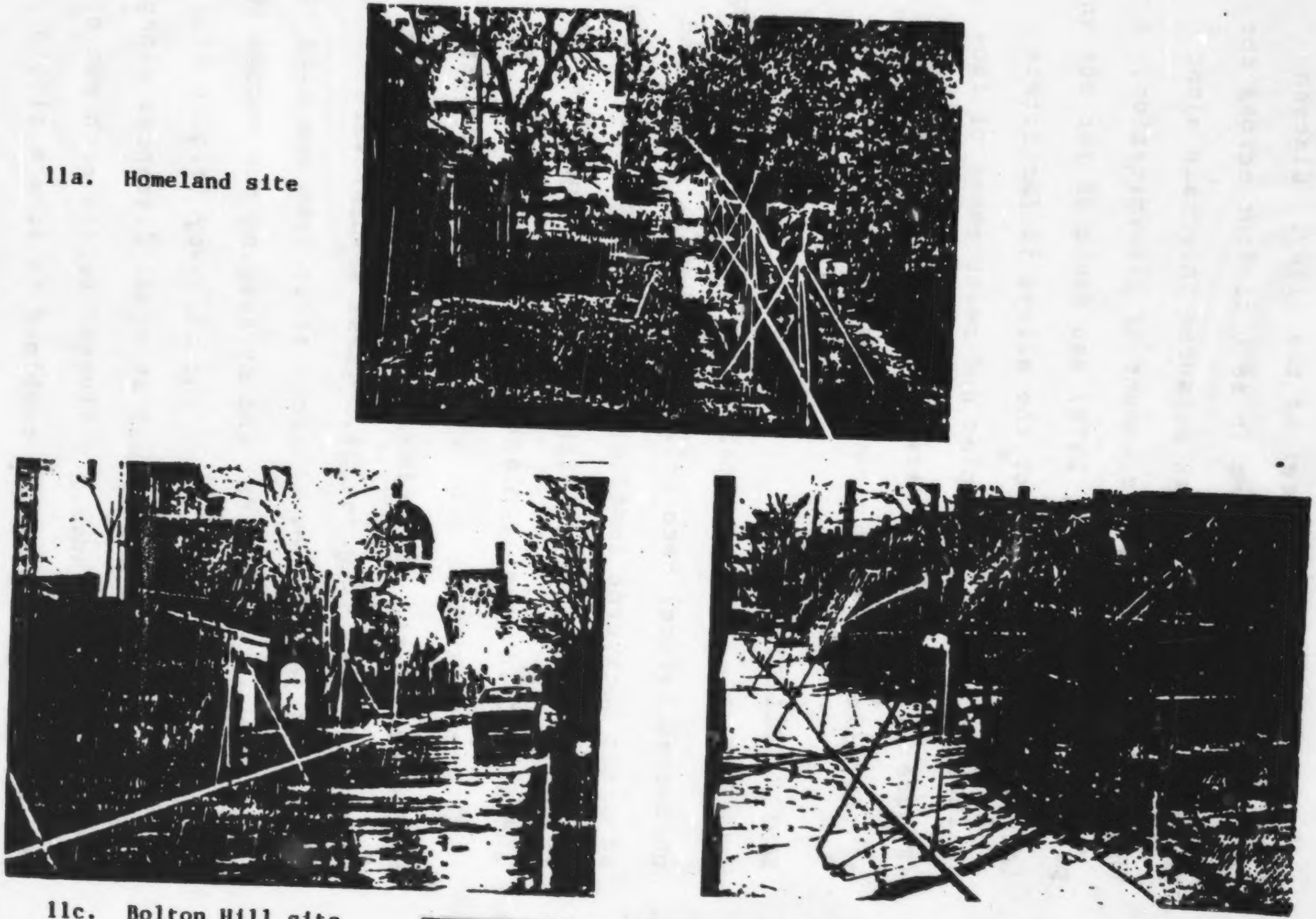

Fire hose
Fire hydrant
Sprinkler
30 ft
Rain gage
20 ft
60 ft
70 ft
90 ft
120ft
150ft
300 ft
180ft
210ft
240ft
270ft
Inlet

Figure 10. Plan view of system



11a. Homeland site

11c. Bolton Hill site

11c. Reservoir Hill site

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Figure 11. Alley flush at 3 study sites

and in line with the center of the alley. Sixteen rainfall gages were placed in sets of four across the swath of the system and at measured intervals along the alley to register the amount of precipitation. A portable H-flume (Grant, 1979) was designed for use in inlets at the lower end of the alleys to facilitate collection of runoff samples and measurement of flow (Figure 12). Sandbags were placed at the lower end of the alleys to divert runoff flow into the inlets.

SAMPLE COLLECTION

Source samples (animal feces, soil, vegetation and uncovered refuse) were collected from the alleys and adjacent backyards immediately before each trial. The samples were collected with sterilized scoops or forceps and stored in sterile plastic bags.

Street surface dust and dirt samples were also collected at the same time using an industrial shop vacuum cleaner (Figure 13). These samples were collected in a narrow (6 inch) strip from one side of the alley (curb to curb) and by keeping the vacuum path aligned at right angles to the alleyway (Figure 14). Three strip samples, spaced at equal distances along the alley, were usually collected before each run of the sprinkler system and combined to form a single composite sample for microbial analysis.

Figure 12. Sample collection at the H-flume.

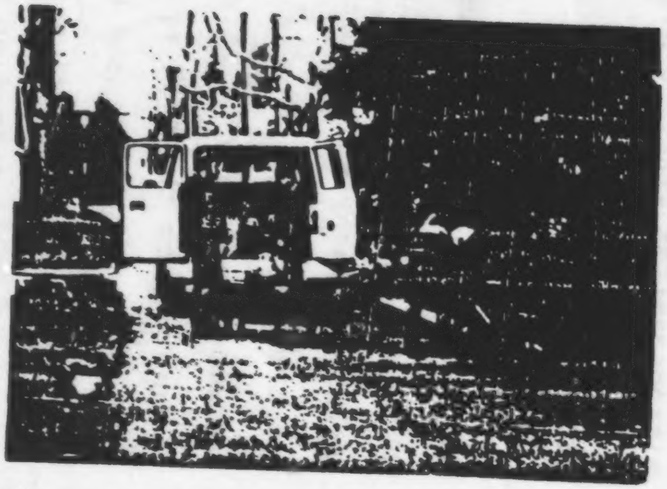

Figure 13. Collection of street surface dust and dirt samples.

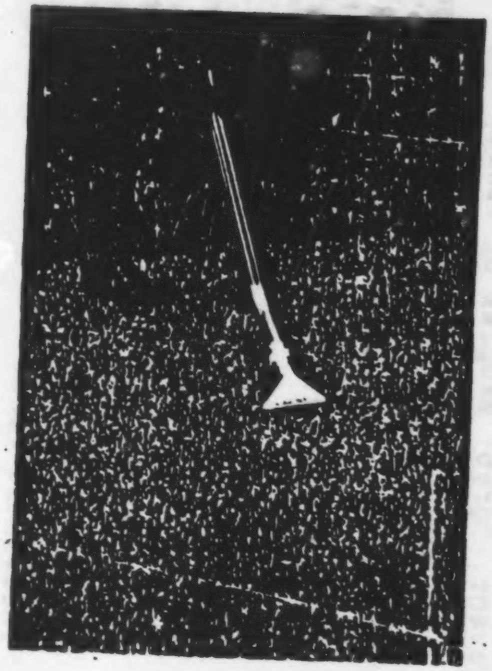

Figure 14. Vacuum path across alleyway.

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With the system turned on to simulate a rainfall event, "grab" samples of runoff water from the alley flush were collected in sterile one-gallon containers at the lower end of the alley by means of the H-flume. Collection of the runoff was carried out throughout the course of the trial, at regular time intervals. This was considered equivalent to taking a flow-composite across the time-period of each run since the flow of runoff at the flume was constant for the greater part of each individual run.

All source and runoff samples were kept on ice immediately after collection and assayed for selected microorganisms within 8 hours from the time of collection.

TESTS FOR RESIDUAL CHLORINE

Before the collection of each "grab" sample, the runoff at the flume was tested for free and combined chlorine by means of a colorimetric procedure using diethyl-p-phenylene diamine (DPD) tablets to ensure that there was no residual chlorine present. Several attempts were made to introduce a sodium thiosulfate solution into the pipe system to neutralize the residual chlorine in the water from the hydrant. Attempts using Venturi set-ups of varying sizes, and various solution-feeding pumps were unsuccessful because of the strong back pressure that occurred in the pipe when the sprinklers were turned on, due to the small holes of the sprinklers acting as limiting orifices to the flow. Sodium thiosulfate was successfully introduced into the system during one trial at the Homeland alley on May 11, 1982. The apparatus used in this instance consisted of a chemical proportioning diaphragm pump connected by a "T" set-up consisting of an aspirator and tygon tubing to 2 points in the pipe system. The diverted flow of water through the tygon tubing helped counteract the effect of backflow through the pipe and facilitated the addition of sodium thiosulfate into the system by the pump. The flow of sodium thiosulfate into the system was measured by negative displacement. The ratio of sodium thiosulfate to estimated residual chlorine from the hydrant was in approximately a 4:1 concentration by weight. Water issuing from the system was tested for chlorine several times throughout the trial and showed no trace of free or combined chlorine. The usual procedure of testing the runoff for residual chlorine before collection was also carried out.

PROCESSING OF SAMPLES

Runoff samples collected in separate bottles at different times throughout each "rainfall" event were processed individually for total coliforms, fecal coliforms and fecal streptococci. A composite of all the runoff samples was made by combining a 2-liter portion of each discrete sample with equal volumes from all the samples collected during the run. This composite was examined for levels of indicator organisms and Salmonella sp.

Source samples were weighed out in 10-gram portions, inoculated into the appropriate media and mechanically shaken or blended for at least 2 minutes to facilitate homogenization. Subsequent ten-fold dilutions were performed for microbiological analysis. All source samples were analyzed for total and fecal coliforms and fecal streptococci and 3-5 samples from each trial were examined for Salmonella sp.

MICROBIOLOGICAL PROCEDURES

Coliform group --

Total coliforms were determined by the multiple tube dilution procedure according to Standard Methods (APHA,1980), using lactose broth with 5 tubes per dilution for the presumptive test. Positive tubes were confirmed on brilliant green lactose broth with 2% bile at 35°C.

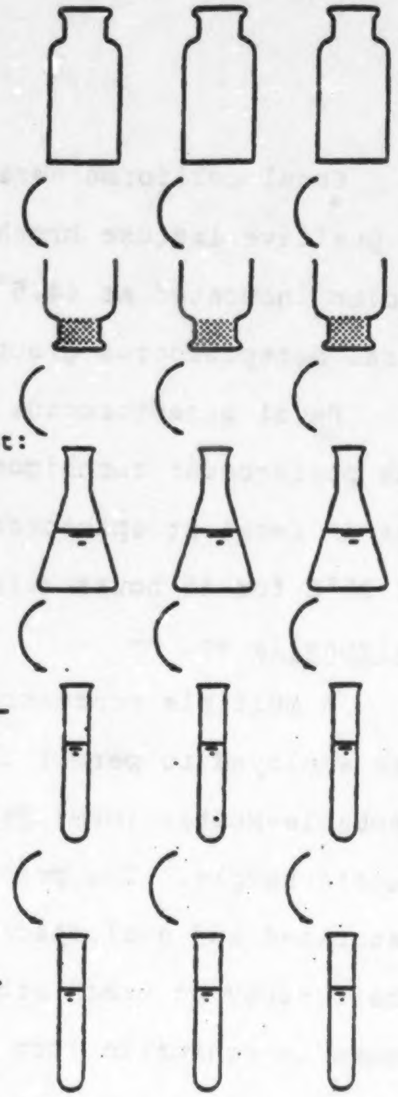
Fecal coliforms were determined by confirmation of positive lactose broth presumptive tubes on EC medium incubated at 44.5°C for 24 hours (APHA,1980).

Fecal Streptococcus group --

Fecal streptococcus densities were determined by the plate-count technique using KF streptococcus agar. The KF fecal streptococcal pour plates were incubated at 35°C for 48 hours before counting (APHA,1980).

Salmonella sp. --

A multiple concentration and enrichment procedure was employed to permit the calculation of a Most-Probable-Number (MPN) *Salmonella* for each composite runoff sample. The procedure followed was that described and evaluated by Olivieri *et al*.(1977) in their study on urban stormwater in Baltimore, and is shown in schematic form in Figure 15. Diatomaceous earth was used for concentration and the enrichment medium employed was dulcitol selenite broth. Dilutions of samples in the enrichment medium were incubated at 40°C, streaked on xylose lysine desoxycholate (XLD) agar for 24 hours, and incubated at 37°C. Streaking was repeated at 48 hours if the primary XLD plates failed to yield typical *Salmonella* colonies (red colonies or red colonies with black centers). Identification of the members of the genus *Salmonella*



Sample

Concentration:
diatomaceous
earth.

Multiple Enrichment:
(1) 10 g filter
plug suspended in
1 x enrichment
medium to 50 ml.

Effective volume:
0.9 x sample.

(2) 10-fold dilution:
5.0 ml of (1) to
5.0 ml 1 x enrichment medium.

Effective volume:
0.09 x sample.

(3) 10-fold dilution:
1.0 ml of (2) to
9.0 ml 1 x enrichment medium.

Effective volume:
0.009 x sample.

(4) Subsequent 10-fold dilutions if
necessary. 1.0 ml
final dilution removed
to waste to maintain
serial dilution.

Figure 15. Schematic - Multiple concentration and enrichment for the MPN determination of *Salmonella*.

was performed according to the schematic given in Figure 16. From the biochemical screen, colonies that were oxidase negative, phenylalanine deaminase negative, utilized malonate, fermented mannitol and lysine decarboxylase positive were used to inoculate triple-sugar-iron agar slants. Bacteria that produced alkaline slants and acid butts as well as hydrogen sulfide and gas were subjected to a serological examination with polyvalent antisera A-I (including Vi) for confirmation.

Source samples were also submitted to the initial enrichment step, with 3 replicate inoculations of 10 grams of sample into dulcitol selenite broth and subsequent 10-fold dilutions. Isolation and identification of Salmonella and calculation of an MPN based on the 3-tube series of dilutions was essentially the same as for the runoff samples.

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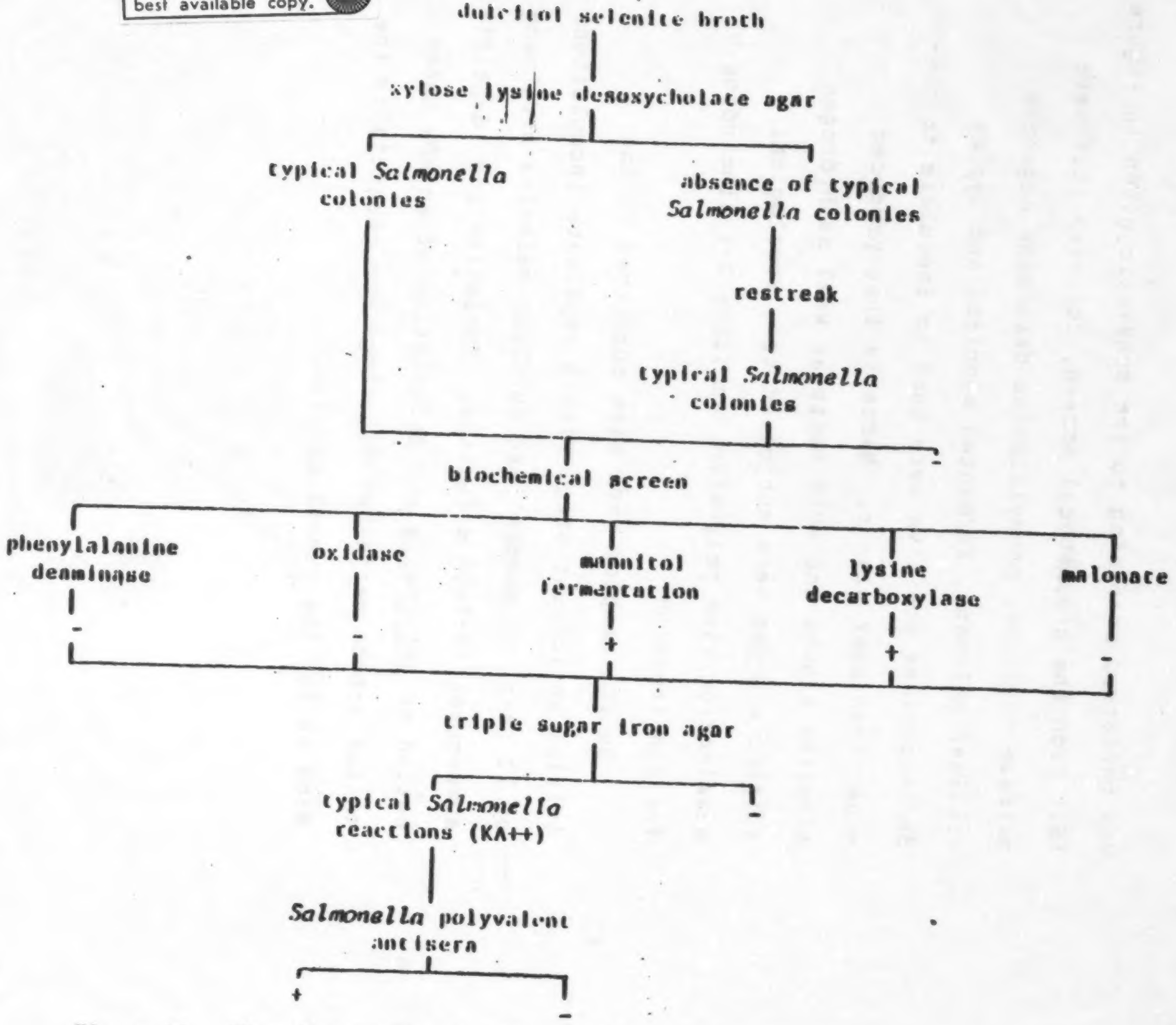
dulcitol selenite broth
xylose lysine desoxycholate agar
typical *Salmonella* colonies
absence of typical *Salmonella* colonies
restreak
typical *Salmonella* colonies
-
biochemical screen
phenylalanine deaminase
-
oxidase
-
mannitol fermentation
+
lysine decarboxylase
+
malonate
-
triple sugar iron agar
typical *Salmonella* reactions (KA++)
-
Salmonella polyvalent antisera
+
-

Figure 16. Flow diagram for the identification of Salmonella.

RESULTS

Simulated rainfall events were conducted and samples were collected from trial runs in the East Baltimore alley in October and early November 1981, and at the three study sites from November to December 1981, and February to June 1982. The study was suspended during the coldest winter month as there was a danger of causing damage to the water mains if the hydrants were turned on in sub-freezing temperatures.

Two trial runs with the sprinkler systems were carried out at the East Baltimore alley and four runs at each alley in Bolton Hill, Reservoir Hill and Homeland. In addition, a natural rainfall event was sampled at the Bolton Hill site. The simulated rainfall runs were conducted weekly or biweekly, and an attempt was made where possible to schedule these runs at least three days following a natural rainstorm event, to minimize any effect that recent rainfall might have on the microbial quality of the samples.

TESTS FOR RESIDUAL CHLORINE

The mean coliform density for samples from the run at the Homeland site where sodium thiosulfate was successfully introduced into the system was compared to the mean density for samples from the other 3 runs at the same site to determine if there was any significant difference in the levels. A similar comparison was made for the data obtained from a natural rain event and 4 experimental runs with the sprinkler system at Bolton Hill. Table 11 shows that there is less than an order of magnitude difference between the means in both sets of comparisons. An analysis of variance of the data comparing mean coliform densities under the different experimental conditions was performed for each site. The F and P values were calculated and indicated no significant differences between the mean densities compared in each case. These are shown below the table.

Tests of the runoff water before sample collection with the DPD colorimetric method never detected any trace of residual chlorine. The aerosolizing nature of the spray from the sprinklers and the presence of bright sunshine on most occasions that the system was run probably played a significant part in the chlorine demand of the water issuing from the hydrant. Once the water strikes the ground, ammonia compounds and various other substances may also cause a further demand on any remaining chlorine residual.

OCCURRENCE AND LEVELS OF MICROORGANISMS

The raw data for the levels of microorganisms in each sample are given in Appendix A (runoff samples)

Table 11. COMPARISONS OF GEOMETRIC MEAN COLIFORM DENSITIES UNDER DIFFERENT EXPERIMENTAL CONDITIONS

		SITE		
		Homeland MPN/100ml		Bolton Hill MPN/100ml
EXPERIMENTAL CONDITIONS	with sodium thiosulfate	4.1E2	natural rainfall	8.1E2
	without sodium thiosulfate	2.5E2	simulated "rainfall"	1.0E3
F and P values from ANOVA of coliform densities, comparing experimental conditions:	F	1.11(NSD)		0.10(NSD)
	P	0.3016		0.7491

NSD No significant difference

and Appendix B (source samples). The levels of indicator organisms for runoff samples are reported as Most Probable Number (MPN) or number/100 ml, and for source samples as MPN or number/gram wet weight. The Salmonella sp. are reported as MPN/10 liters for runoff samples and MPN/gram for source samples. The denominator for the Salmonella data is thus a hundred times that for the indicator data in the case of runoff samples.

RUNOFF SAMPLES

The geometric mean densities and ranges for total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) assayed for runoff samples from each alley location are given in Table 12. The geometric mean indicator densities for the East Baltimore alley were very high (TC-4,700,000/100 ml; FC-1,800,000/100 ml; FS-3,500,000/100 ml), and exceeded those for all the 3 study sites by several orders of magnitude. The Reservoir Hill alley had the highest levels of these microorganisms (TC-110,000/100 ml; FC-49,000/100 ml; FS-6,500/100 ml), with total and fecal coliform densities exceeding those from Bolton Hill (TC-1,000/100 ml; FC-170/100 ml; FS-3,600/100 ml) by 2 orders of magnitude, and those from Homeland (TC-280/100 ml; FC-33/100 ml;

Table 12. GEOMETRIC MEAN (GM) AND RANGE (R) OF LEVELS OF INDICATOR ORGANISMS IN RUNOFF SAMPLES COLLECTED IN TEST ALLEYS

Site	Number of Samples		Total Coliform MPN/100ml	Fecal Coliform MPN/100ml	Fecal Strep No./100ml
Homeland	26	GM	2.8E2	3.3E1	7.9E2
		R	3.9E1-1.7E3	2.0E0-2.3E2	2.0E2-6.2E3
Bolton Hill	26	GM	1.0E3	1.7E2	3.6E3
		R	2.7E1-7.9E3	2.0E0-4.9E3	3.2E2-2.9E4
Reservoir Hill	24	GM	1.1E5	4.9E4	6.5E3
		R	2.3E4-7.1E5	4.0E3-3.3E5	4.1E2-6.0E5
East Baltimore	9	GM	4.7E6	1.8E6	3.5E6
		R	1.3E5-4.9E8	4.9E4-3.3E8	1.1E6-1.0E7

F and P values from ANOVA of indicator densities, comparing sites:		Total Coliform	Fecal Coliform	Fecal Strep
	F	136.07**	131.38**	60.75**
	P	0.0001	0.0001	0.0001

** Significant difference at 1%

FS-790/100 ml) by 3 orders of magnitude. Indicator levels from the Bolton Hill alley were in turn greater than levels from the Homeland location by approximately one order of magnitude.

SOURCE SAMPLES

The geometric mean densities and ranges for indicator organisms for 6 categories of source samples are shown in Table 13. Of those 6 categories, animal feces is clearly seen to have the highest levels of coliform bacteria and fecal streptococci. The levels of fecal coliforms in dog feces and rat feces were 830,000/gram and 520,000/gram respectively. The mean fecal coliform density for uncovered refuse samples was 500/gram. There does not appear to be a great disparity between indicator levels for street surface dust and dirt, soil and vegetation samples. The mean densities of fecal coliforms for these 3 categories were all less than 100/gram.

The F tests from analysis of variance of the data indicated significant differences between the mean bacterial densities from the 6 categories. The levels of microorganisms in individual samples from each category covered a wide range, often with several orders of magnitude difference between minimum and maximum density levels.

Table 13. GEOMETRIC MEAN (GM) AND RANGE (R) OF LEVELS OF INDICATOR ORGANISMS IN SOURCE SAMPLES (COMPARED BY CATEGORY)

Sample Category	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Street Surface	9	GM	1.5E3	4.7E1	1.8E3
		R	7.1E1-7.9E4	<2.0E0-7.9E3	1.3E2-1.3E4
Dog feces	38	GM	2.0E6	8.3E5	2.6E7
		R	7.9E2-1.3E9	1.1E2-1.3E9	2.7E4-2.9E9
Rat feces	4	GM	1.4E6	5.2E5	2.9E7
		R	7.1E5-4.9E6	1.3E5-2.2E6	1.3E7-4.0E7
Uncovered Refuse	6	GM	1.6E3	5.0E2	2.2E4
		R	2.0E0-1.3E7	<2.0E0-1.1E6	1.2E3-7.6E6
Soil	33	GM	4.8E2	5.2E1	1.1E3
		R	<2.0E0-2.2E6	<2.0E0-3.2E5	<1.0E1-5.4E6
Vegetation	28	GM	4.7E2	5.1E1	1.8E3
		R	<2.0E0-1.7E7	<2.0E0-2.0E5	5.0E0-5.4E7

+ wet weight

F and P values from ANOVA of indicator densities, comparing source sample categories:		Total Coliform	Fecal Coliform	Fecal Strep
	F	28.61**	44.83**	55.44**
	P	0.0001	0.0001	0.0001

** Significant difference at 1%

The geometric mean densities of coliform microorganisms and fecal streptococci in source samples belonging to various categories were also compared for the individual sites, as shown in Table 14. This more detailed breakdown not only allows a comparison of the levels of indicator bacteria among different categories of samples from the same site, but also provides a means of ascertaining whether source samples from the same category give significantly different levels when collected from different sites. For each individual site, animal feces remains the category that has the highest bacterial levels.

The results of the F tests for significant differences between the mean bacterial densities of samples from the different sites are shown below each data category. Significant differences between coliform and fecal streptococcal densities were found for soil and vegetation samples from the 4 alleyways, with the East Baltimore alley having the highest bacterial densities (>15,000/gram) and the Homeland alley the lowest (<200/gram) in both categories. Coliform densities for uncovered refuse from two sites were significantly different. In the case of animal feces, significant differences were obtained between densities of fecal streptococci. No significant differences were found for coliforms or fecal streptococci for the street surface dust and dirt samples.

Table 14. GEOMETRIC MEAN (GM) AND RANGE (R) OF LEVELS OF INDICATOR ORGANISMS IN SOURCE SAMPLES (COMPARED BY SAMPLE CATEGORY AND SITE)

SAMPLE CATEGORY: STREET SURFACE DUST AND DIRT

Site	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Homeland	2	GM	2.2E2	1.2E1	5.8E2
		R	7.1E1-1.3E3	<2.0E0-1.1E2	1.3E2-3.0E3
Bolton Hill	3	GM	6.3E3	2.0E1	2.9E3
		R	2.3E3-1.7E4	2.0E0-2.0E2	6.3E2-1.3E4
Reservoir Hill	4	GM	7.8E3	5.2E2	5.9E3
		R	4.6E2-7.9E4	1.3E2-7.9E3	1.6E3-1.3E4

+ wet weight

F and P values from ANOVA of indicator densities, comparing sites:		Total Coliform	Fecal Coliform	Fecal Strep
	F	3.78(NSD)	2.56(NSD)	2.03(NSD)
	P	0.0867	0.1568	0.2119

SAMPLE CATEGORY: DOG FECES

Site	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Homeland	7	GM	1.1E7	9.3E5	1.6E7
		R	6.5E4-1.8E8	2.0E4-1.3E8	8.7E5-2.9E9
Bolton Hill	14	GM	6.0E5	4.4E5	5.0E6
		R	7.9E2-2.3E8	1.1E2-2.3E8	2.7E4-2.1E8
Reservoir Hill	12	GM	2.0E6	1.4E6	1.9E8
		R	2.3E4-1.3E9	2.3E4-1.3E9	5.9E6-2.7E9
East Baltimore	5	GM	4.4E6	1.3E6	4.6E7
		R	3.3E5-7.9E7	3.3E5-7.9E7	2.0E7-2.2E8

F and P values from ANOVA of indicator densities, comparing sites:		Total Coliform	Fecal Coliform	Fecal Strep
	F	1.12(NSD)	0.22(NSD)	5.65**
	P	0.3545	0.8842	0.0030

NSD No significant difference

** Significant difference at 1%

Table 14 - continued

SAMPLE CATEGORY: SOIL

Site	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Homeland	12	GM	4.9E1	7.2E0	7.8E1
		R	<2.0E0-1.7E3	<2.0E0-4.5E1	<1.0E1-4.5E3
Bolton Hill	11	GM	4.9E2	4.4E1	1.0E3
		R	1.3E1-4.5E3	<2.0E0-2.0E2	3.5E2-5.0E3
Reservoir Hill	6	GM	8.9E2	8.9E1	6.5E3
		R	3.3E1-1.3E4	<2.0E0-3.3E2	6.0E2-8.5E4
East Baltimore	4	GM	1.8E5	1.5E4	3.8E5
		R	3.3E4-2.2E6	2.0E2-3.2E5	3.5E4-4.1E6

F and P values from ANOVA of indicator densities, comparing sites:		Total Coliform	Fecal Coliform	Fecal Strep
	F	16.44**	18.83**	27.62**
	P	0.0001	0.0001	0.0001

SAMPLE CATEGORY: VEGETATION

Site	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Homeland	9	GM	1.0E2	1.5E1	1.8E2
		R	<2.0E0-1.8E3	<2.0E0-1.1E2	5.0E0-3.0E3
Bolton Hill	10	GM	3.1E2	2.2E1	1.1E3
		R	4.5E0-1.3E4	<2.0E0-7.9E2	1.0E1-7.1E4
Reservoir Hill	6	GM	3.4E2	4.8E1	7.1E3
		R	7.8E1-1.1E3	1.4E1-2.8E2	2.1E3-7.8E4
East Baltimore	3	GM	3.5E5	3.9E4	5.8E5
		R	4.9E4-1.7E7	6.2E3-2.0E5	3.3E4-5.4E7

F and P values from ANOVA of indicator densities, comparing sites:		Total Coliform	Fecal Coliform	Fecal Strep
	F	6.73**	14.06**	6.13**
	P	0.0019	0.0001	0.0019

** Significant difference at 1%

Table 14 - continued

SAMPLE CATEGORY: RAT FECES

Site	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Reservoir Hill	1		9.6E5	3.3E5	1.3E7
East Baltimore	3	GM	1.5E6	6.0E5	3.7E7
		R	7.1E5-4.9E6	1.3E5-2.2E6	3.4E7-4.0E7
F and P values from ANOVA of indicator densities, comparing sites:			Total Coliform	Fecal Coliform	Fecal Strep
		F	0.18(NSD)	0.14(NSD)	110.46**
		P	0.7153	0.7465	0.0089

SAMPLE CATEGORY: UNCOVERED REFUSE

Site	Number of Samples		Total Coliform MPN/gram+	Fecal Coliform MPN/gram+	Fecal Strep No./gram+
Reservoir Hill	4	GM	5.9E1	2.6E1	4.5E3
		R	2.0E0-1.1E4	<2.0E0-6.2E2	1.2E3-9.1E4
East Baltimore	2	GM	1.2E6	1.9E5	5.5E5
		R	1.1E5-1.3E7	3.1E4-1.1E6	4.0E4-7.6E6
F and P values from ANOVA of indicator densities, comparing sites:			Total Coliform	Fecal Coliform	Fecal Strep
		F	10.11*	18.14*	4.71(NSD)
		P	0.0336	0.0131	0.0957

NSD No significant difference
* Significant difference at 5%
** Significant differnce at 1%

SALMONELLA

The results of Salmonella assays of composite runoff samples and selected source samples are shown in Table 15. Salmonella could not be detected in any of the runoff, soil, vegetation or street surface samples at the lower sensitive limit of the assay (MPN <1/100 ml for runoff samples and <0.03/gram for source samples). Out of 32 animal fecal samples assayed for Salmonella, 6 were found to be positive (19%) with a geometric mean density of 1.6 Salmonella/gram and a range of 0.04/gram to 21/gram. Of these 6 samples, 3 were from the Bolton Hill alley and 3 from the Reservoir Hill alley. None of the fecal samples collected from the Homeland site and assayed for Salmonella were positive for this pathogen.

VARIATION WITH FLOW

The variation in levels of microoganisms in runoff samples collected during the sampling period for individual runs are shown in Figures 17 to 27. As can be seen, there were only slight differences between indicator levels for each individual "grab" sample. The pattern of flow at the point of sample collection is also depicted for the time period of each run. After a sharp initial rise, the flow generally remained constant for the greater part of the time course of the event, hence collection of runoff samples at regular

Table 15. OCCURRENCE OF *SALMONELLA* IN RUNOFF AND SOURCE SAMPLES

Sample	Total No. of Samples	No. Samples Assayed for *Salmonella*	No. Samples Positive for *Salmonella*	Percen Positi
Runoff	13	13	0	0
Dog Feces	38	32	6	19
Soil	33	11	0	0
Vegetation	28	11	0	0
Street Surface dust and dirt	9	9	0	0

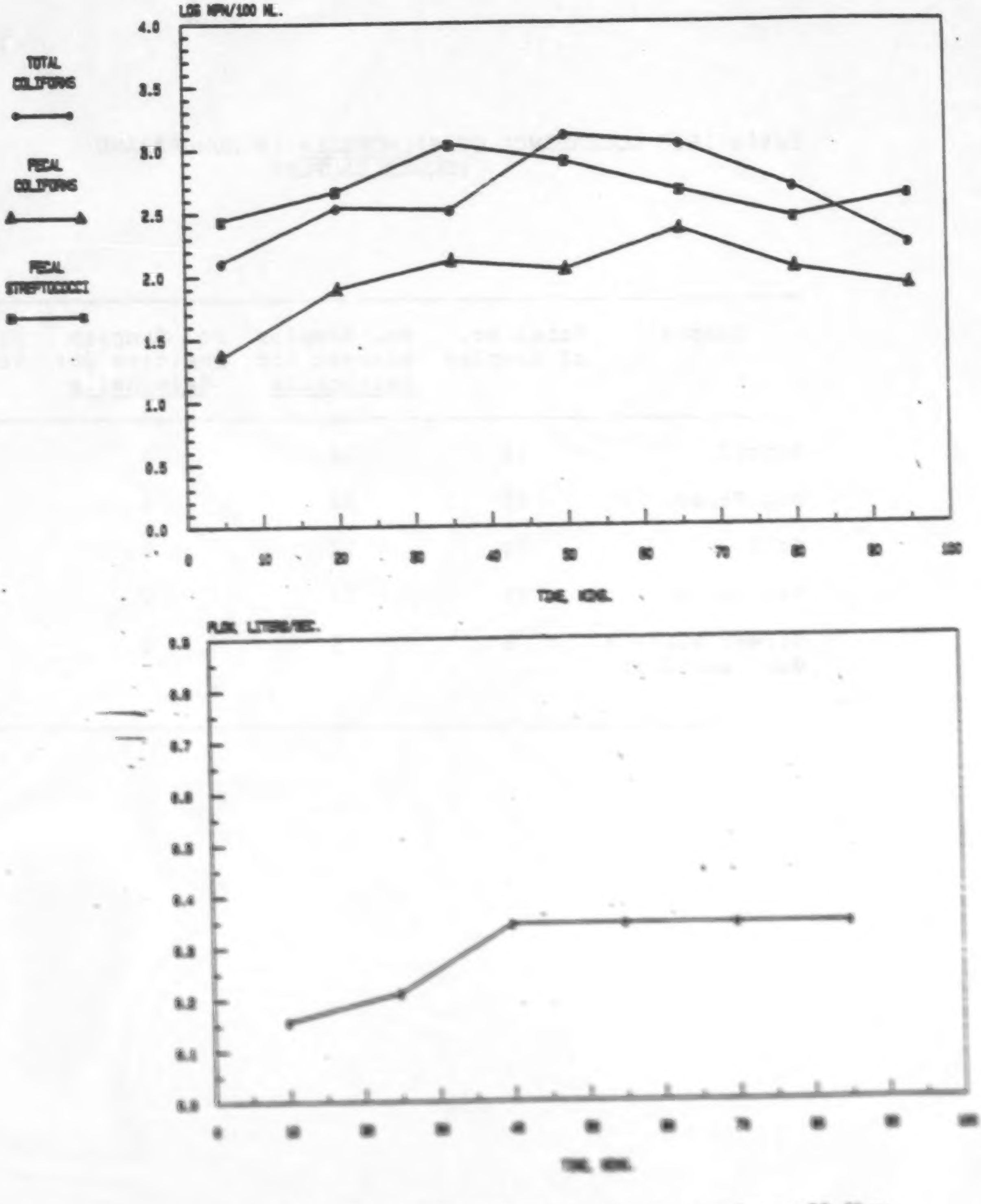

Figure 17. Levels of indicator organisms and runoff flow during the sampling period. Run of 12/11/81, Homeland site.
Amount of "rainfall" = 1.0 cm. (0.4 in.)

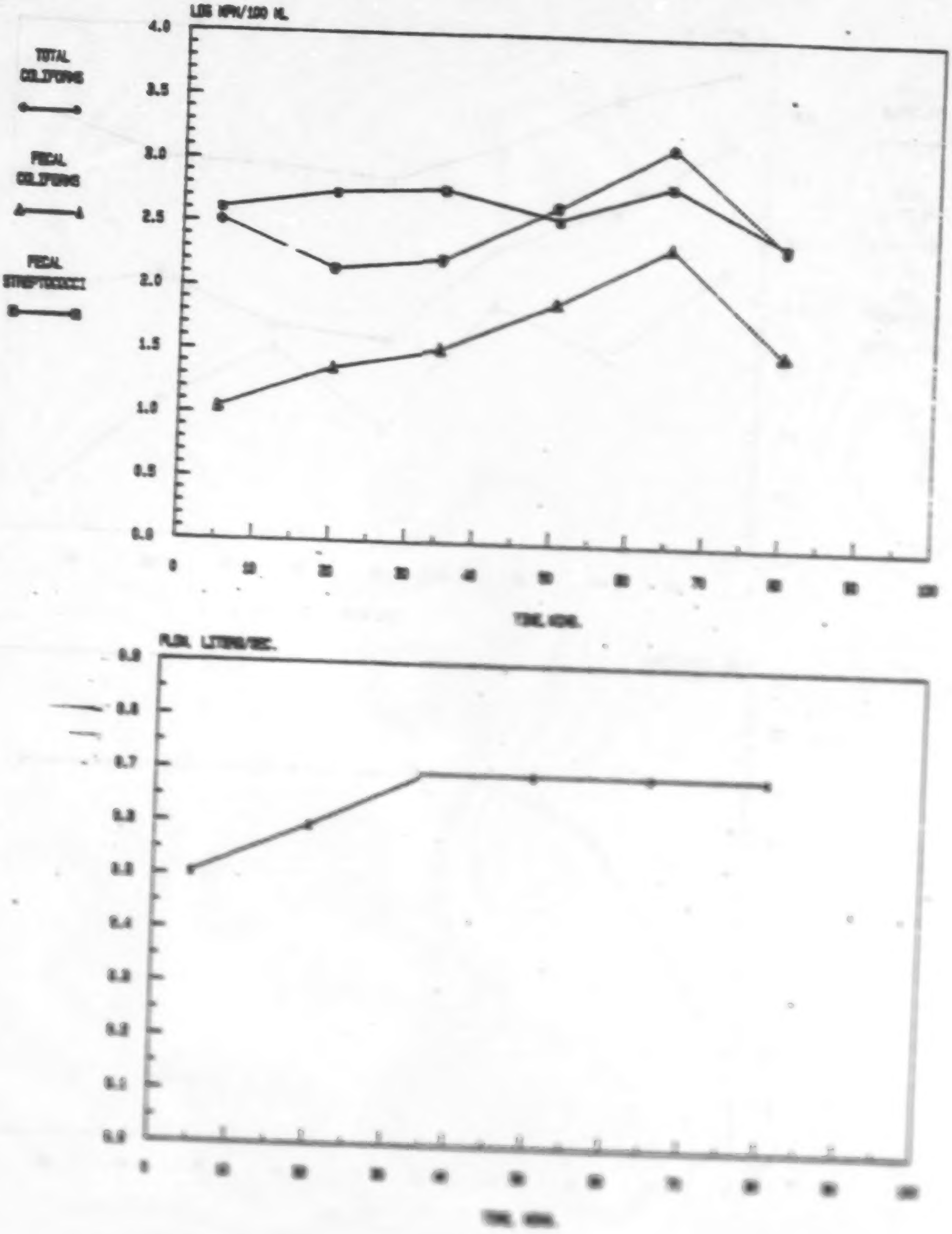

Figure 18. Levels of indicator organisms and runoff flow during the sampling period, Run of 3/23/82, Homeland sit.
Amount of "rainfall" = 1.3 cm. (0.5 in.)

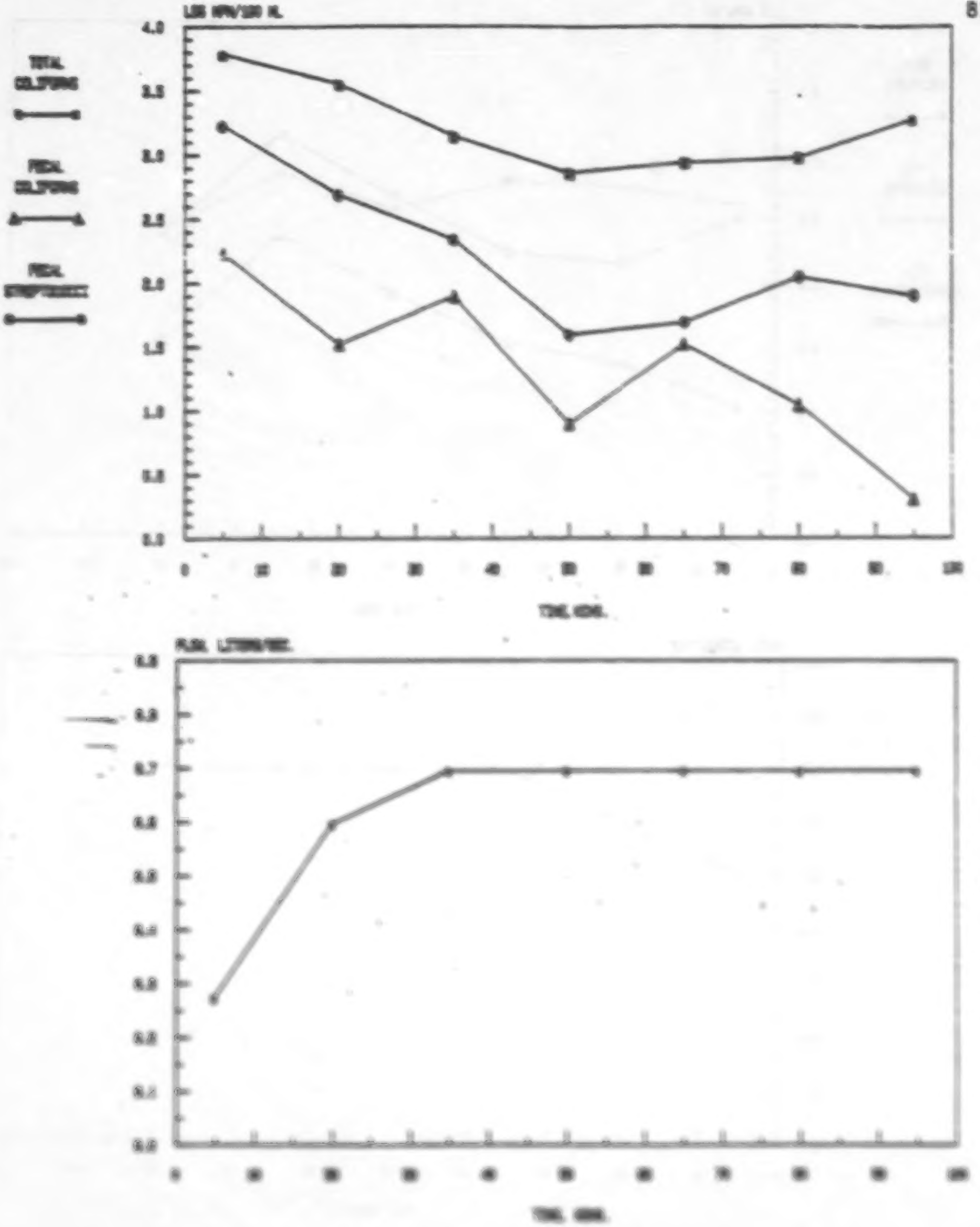

Figure 19. Levels of indicator organisms and runoff flow during the sampling period, Run of 5/4/82, Homeland site.
Amount of "rainfall" = 1.3 cm. (0.5 in.)

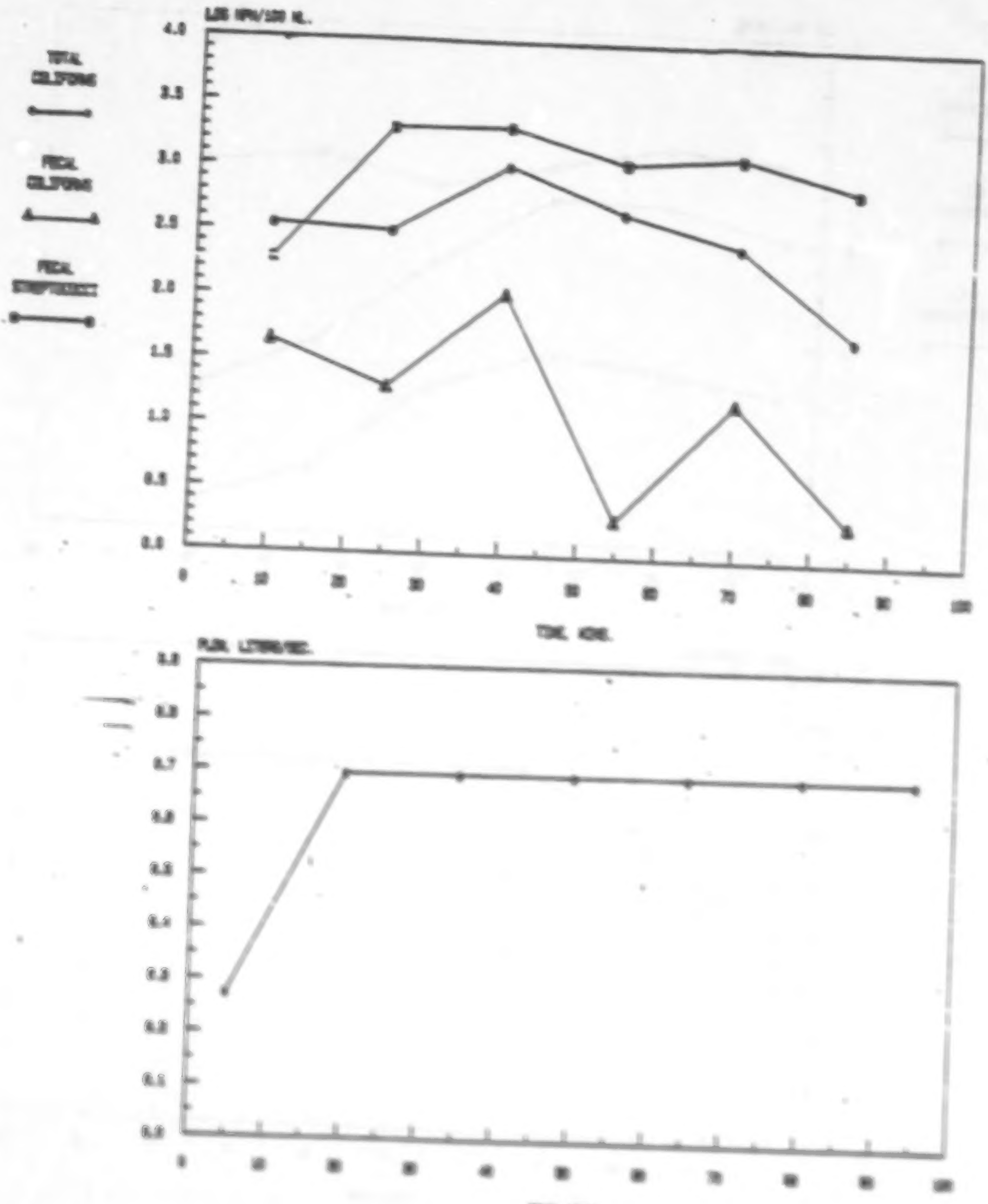

Figure 20. Levels of indicator organisms and runoff flow during the sampling period. Run of 5/11/82, Homeland site.
Amount of "rainfall" = 1.3 cm. (0.5 in.)

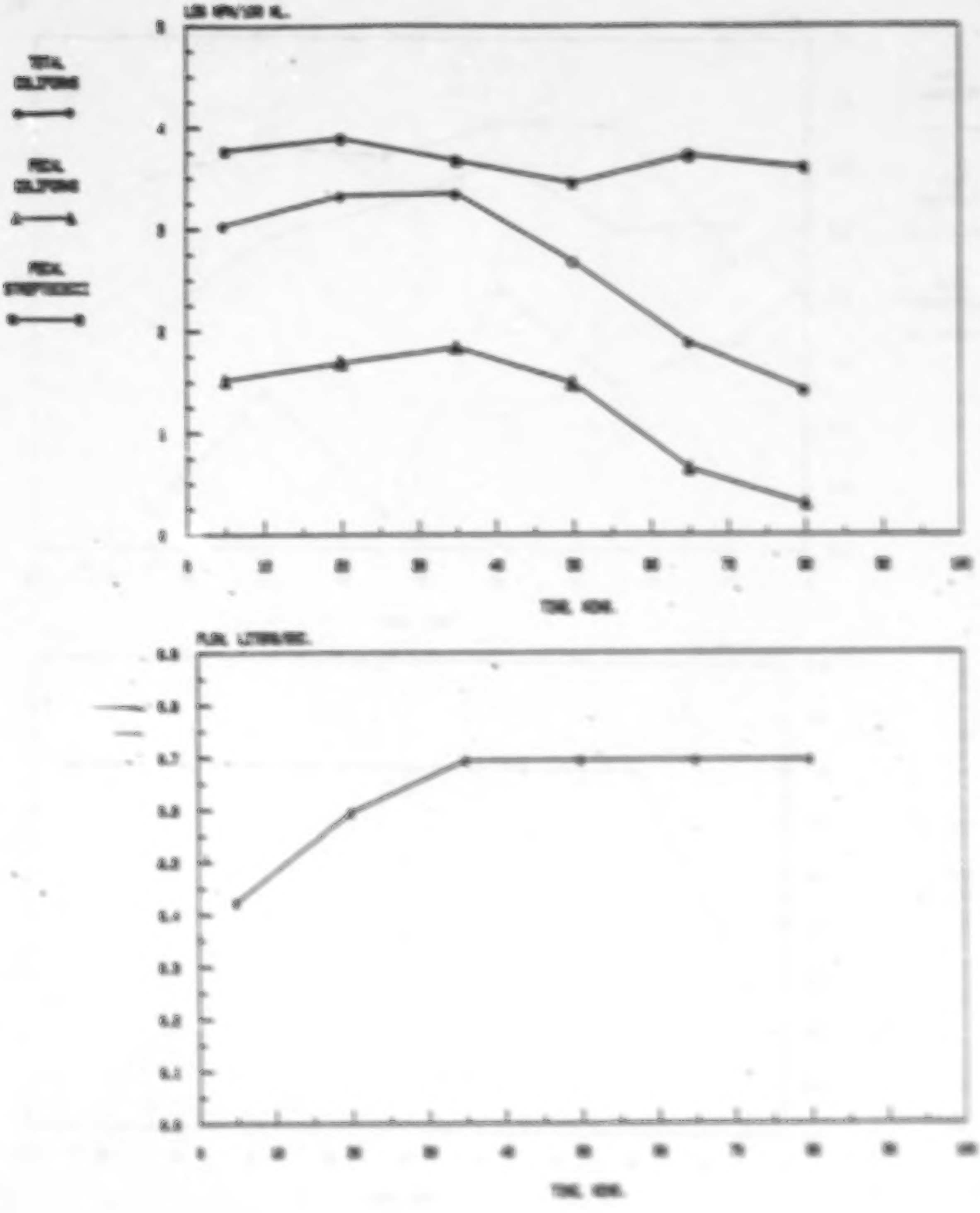

Figure 21. Levels of indicator organisms and runoff flow during the sampling period, Run of 2/28/82, Bolton Hill site.
Amount of "rainfall" = 1.3 cm. (0.5 in.)

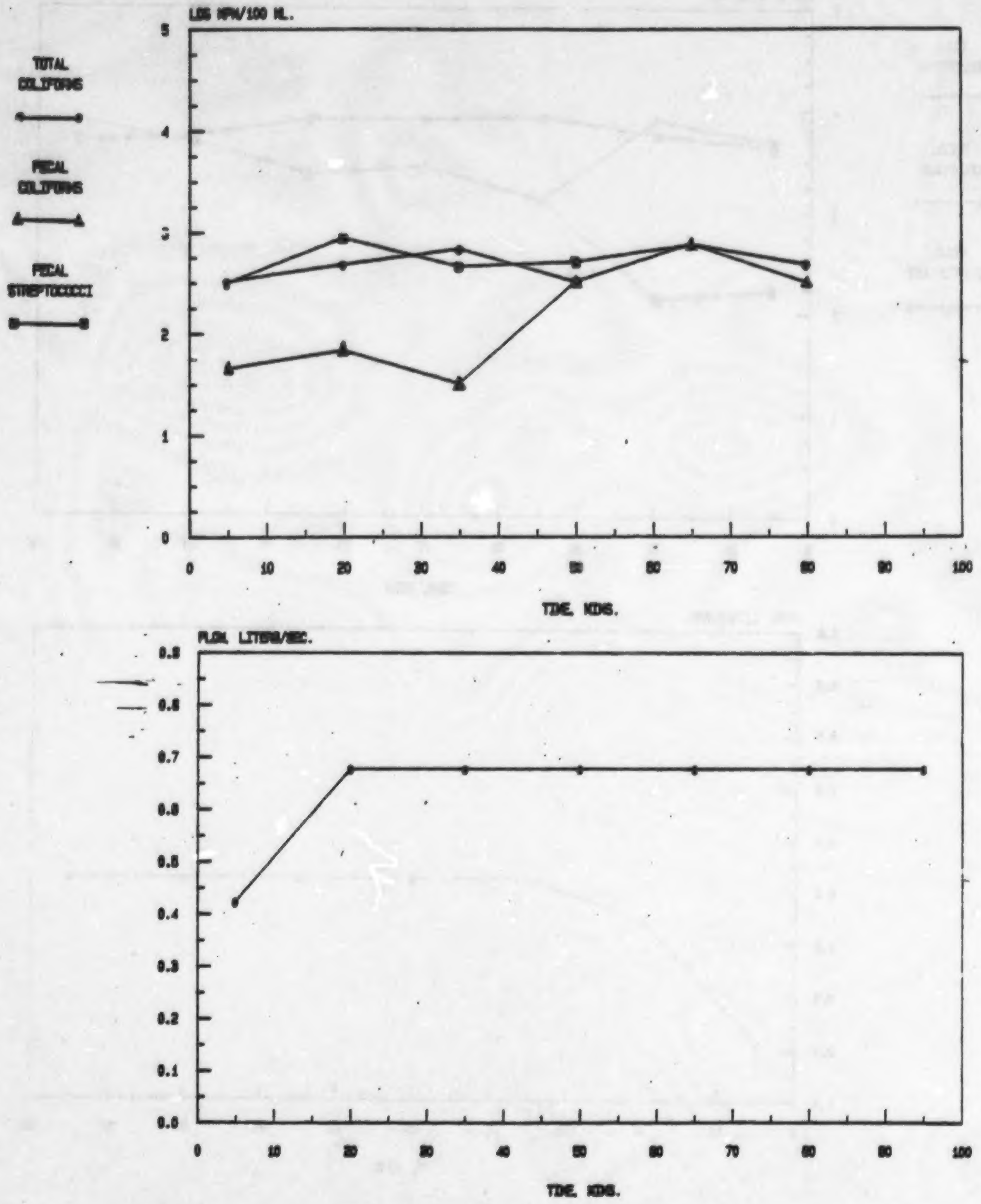

Figure 22. Levels of indicator organisms and runoff flow during the sampling period, Run of 4/21/82, Bolton Hill site.
Amount of "rainfall" = 1.3 cm. (0.5 in.)

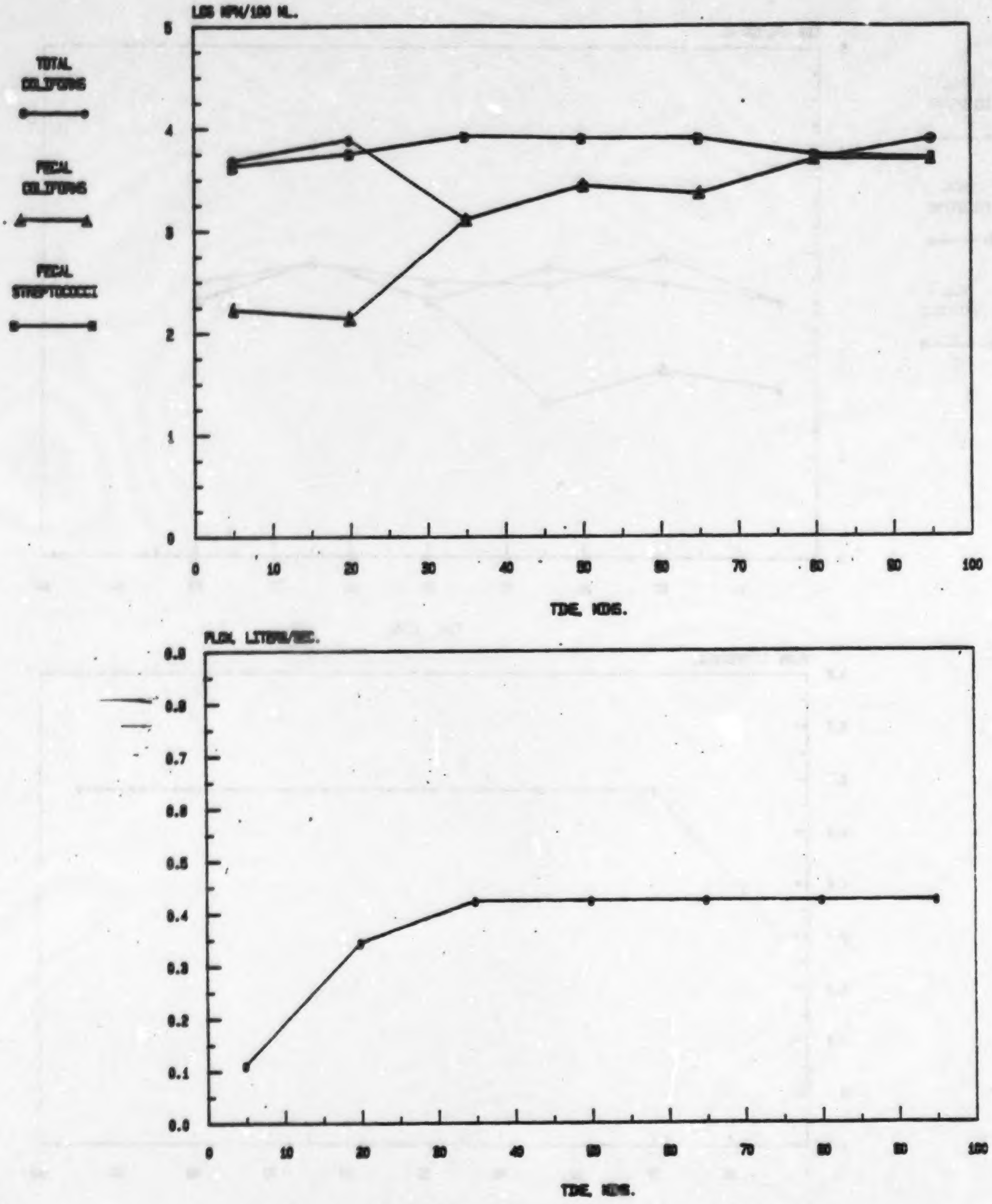

Figure 23. Levels of indicator organisms and runoff flow during the sampling period, Run of 5/18/82, Bolton Hill site.
Amount of "rainfall" = 1.0 cm. (0.4 in.)

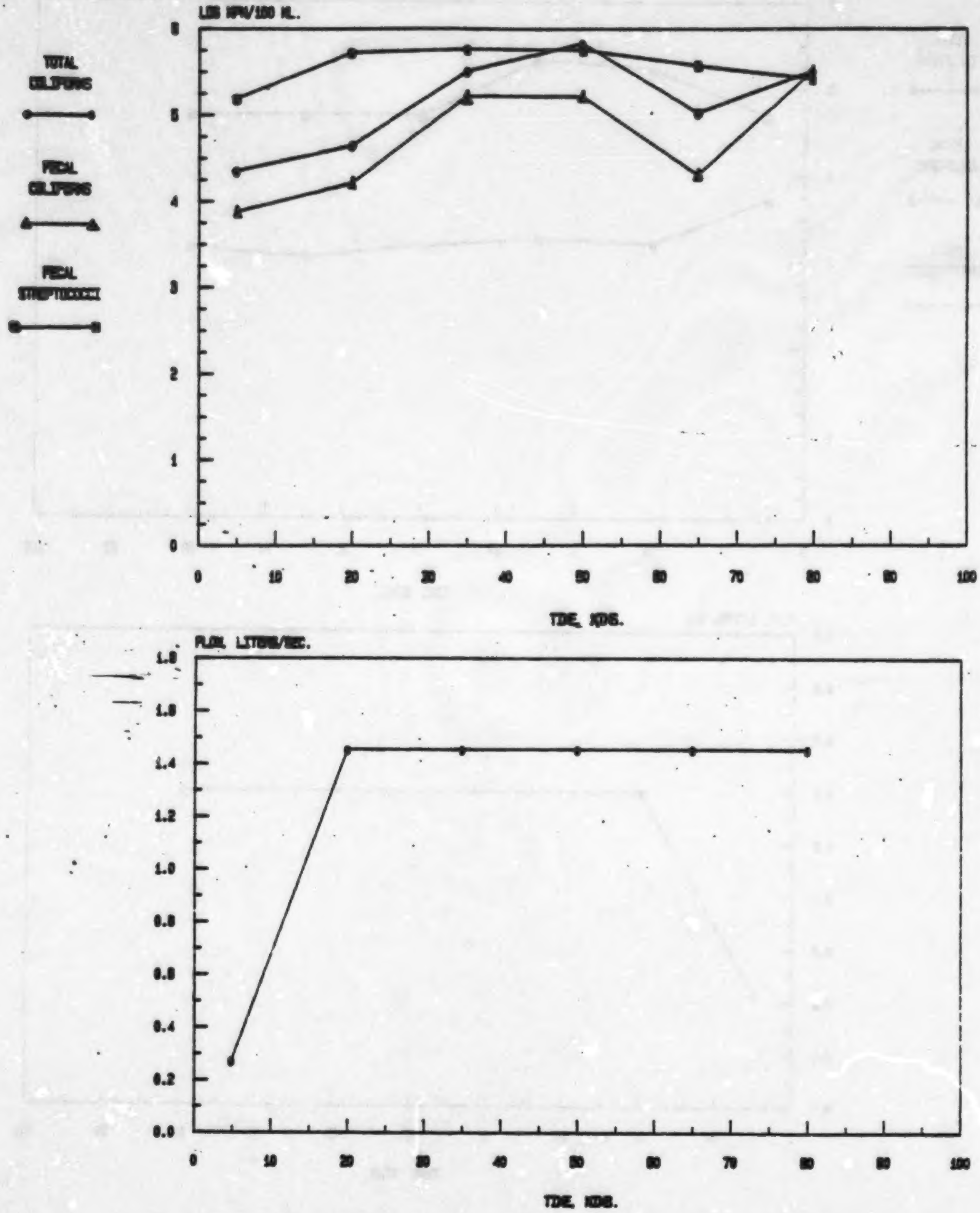

Figure 24. Levels of indicator organisms and runoff flow during the sampling period, Run of 11/24/81, Reservoir Hill site.
Amount of "rainfall" = 2.3 cm. (0.9 in.)

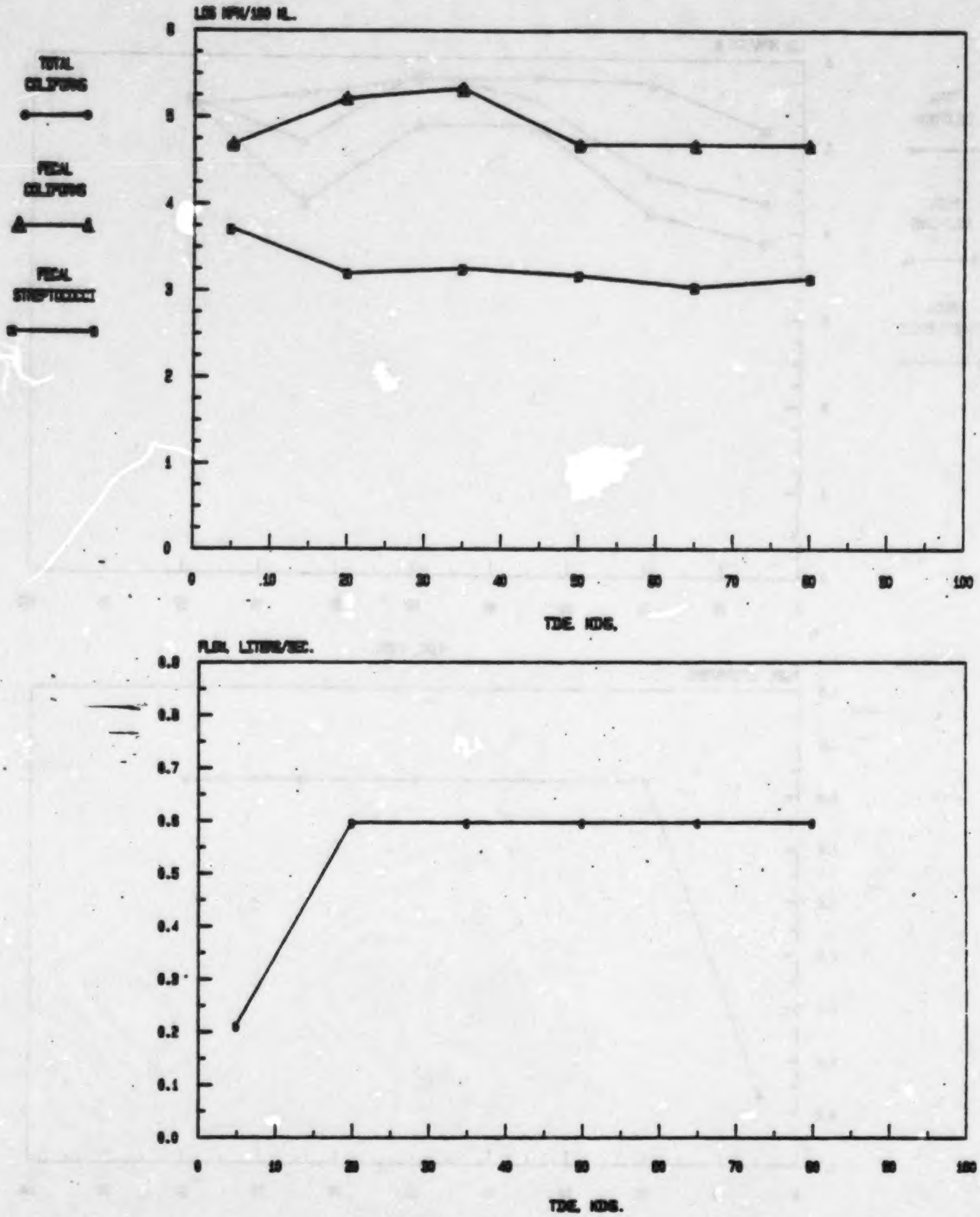

Figure 25. Levels of indicator organisms and runoff flow during the sampling period, Run of 4/16/82, Reservoir Hill site.
Amount of "rainfall" = 1.5 cm. (0.6 in.)

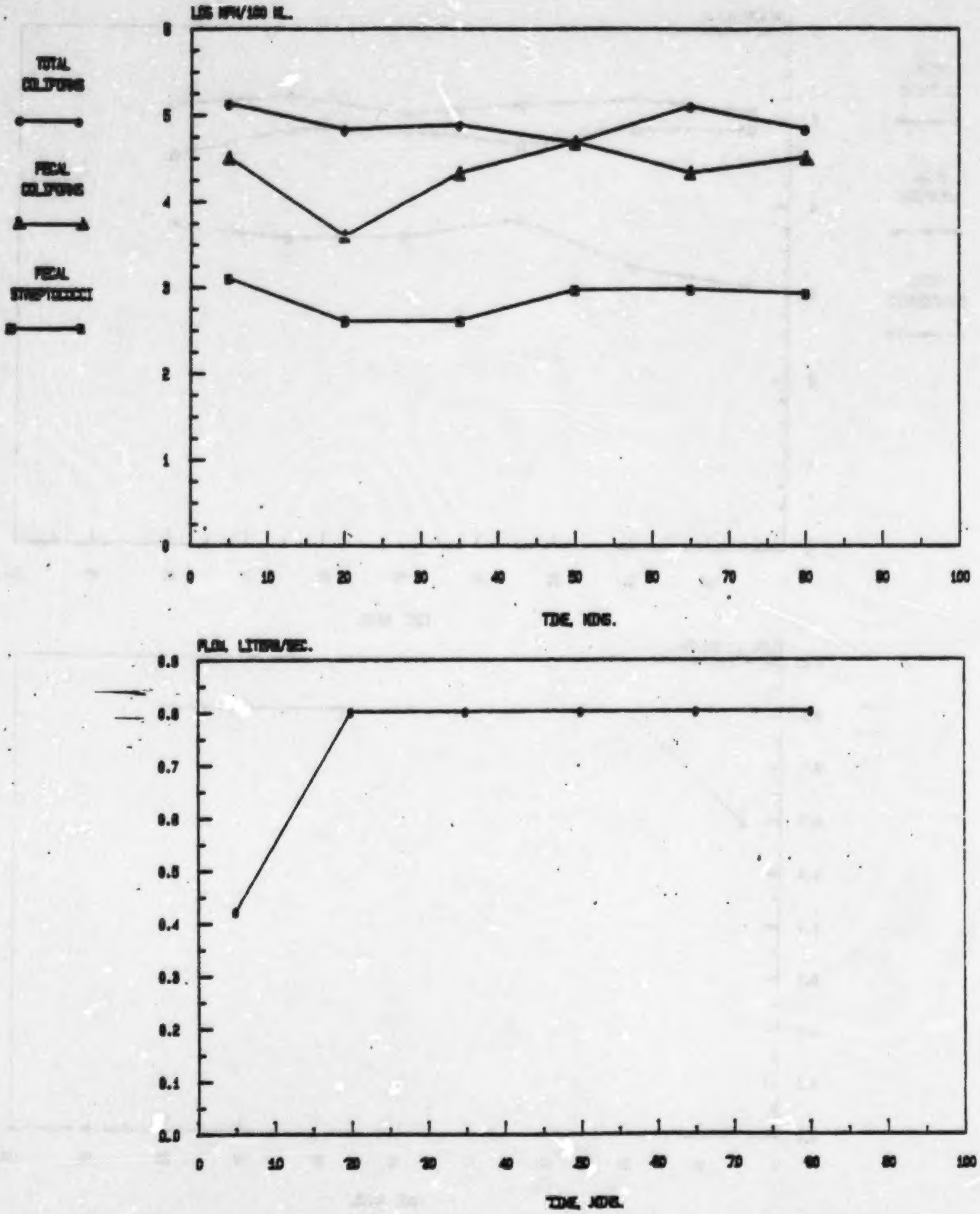

Figure 26. Levels of indicator organisms and runoff flow during the sampling period, Run of 4/23/82, Reservoir Hill site.
Amount of "rainfall" = 2.0 cm. (0.8 in.)

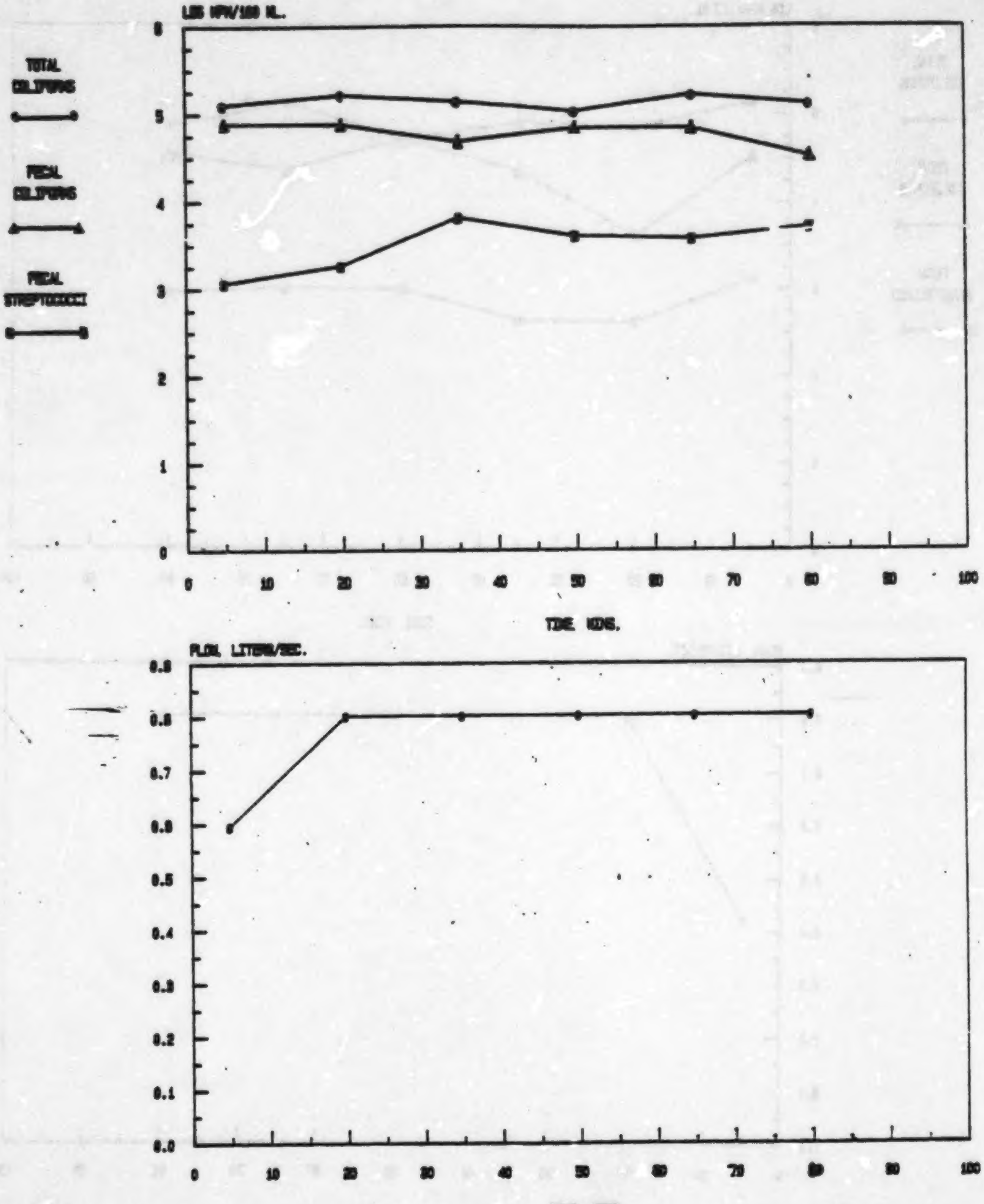

Figure 27. Levels of indicator organisms and runoff flow during the sampling period, Run of 4/30/82, Reservoir Hill site.
Amount of "rainfall" = 2.0 cm. (0.8 in.)

time intervals was considered equivalent to sampling a flow-composite across the time period of each run. This assumption was further substantiated by statistical analysis of indicator densities in discrete and composite samples for individual runs, where the F-tests for all runs gave no significant differences between the mean densities for discrete samples and composite sample densities (Appendix C).

The amount of "rainfall" generated for each event is indicated below each figure. There does not appear to be an initial flushing effect on the levels on indicator bacteria in the runoff samples. Little correlation was found between the densities of indicator microorgnaisms and flow throughout each run.

MICROBIAL LOADS

Since runoff flows were almost constant for individual trials for each drainage area, the mean bacterial loadings could be calculated. The microbial load is obtained from the product of the MPN or number/100 ml and the flow of runoff determined at the H-flume in ml/second. The distinct difference between levels of microorganisms in runoff samples from the different sites can also be seen in Table 16, which compares their mean indicator bacteria loadings (MPN or number/second). The F values from the comparison of mean

Table 16. MEAN INDICATOR BACTERIAL LOADS IN RUNOFF SAMPLES

Site	Number of Samples	Total Coliform MPN/sec	Fecal Coliform MPN/sec	Fecal Strep No./sec
Homeland	26	2.4E3	3.9E2	5.7E3
Bolton Hill	19	9.1E3	4.3E3	2.0E4
Reservoir Hill	24	1.5E6	7.4E5	1.5E6
East Baltimore++	9	8.5E8	3.7E8	3.8E7

++ Flows were not measured for preliminary trial runs. For calculations of bacterial loads runoff flows were estimated from total rainfall x area covered by system ÷ time system was turned on, flows assumed to be constant and 50% impervious area.

F and P values from ANOVA of indicator loads, comparing sites:		Total Coliform	Fecal Coliform	Fecal Strep
	F	8.69**	6.30**	52.64**
	P	0.0001	0.0008	0.0001

** Significant difference at 1% level

loads between sites were significant for coliform and fecal streptococcal loads. The mean fecal coliform load per second for the Reservoir Hill alley (74,000/second) was 2 orders of magnitude greater than the load from the Bolton Hill site (4,300/second) , and 3 orders of magnitude higher than that from the Homeland location (390/second). The estimated fecal coliform load from the East Baltimore alley (370,000,000/second) exceeded the loads from the other 3 sites by several orders of magnitude.

ANTECEDENT RAINFALL

The effect of previous rainfall prior to the time of sampling on the microbial quality of the runoff was evaluated for each of the 3 study locations. The levels of fecal coliforms were compared to the antecedent of number of days since the last rainfall, as shown in Figures 28 to 30. Antecedent rainfall, in days, appears to have little effect on the fecal coliform densities in the runoff samples. Similar results were obtained with total coliform and fecal streptococcal densities.

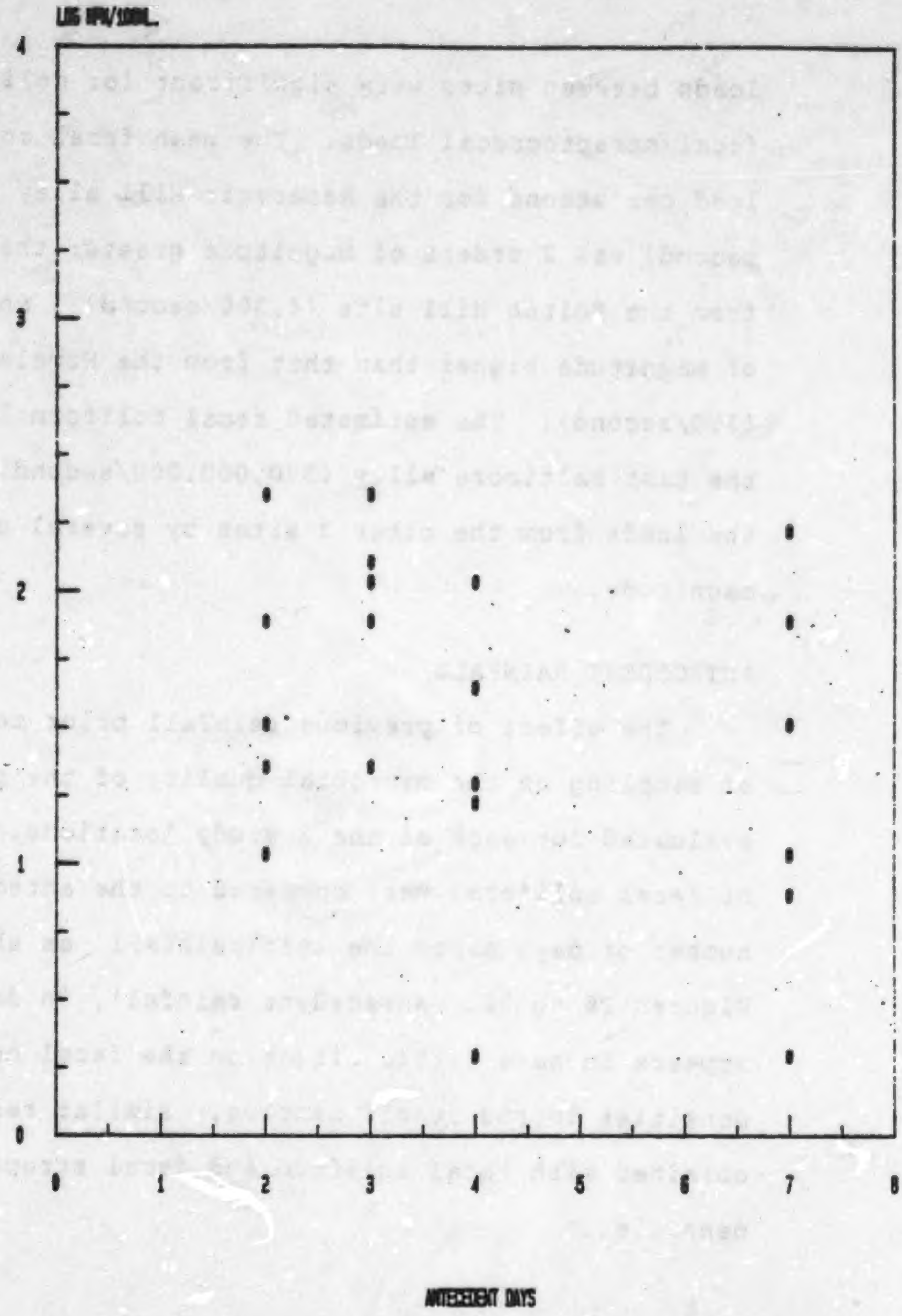

Figure 28. The effect of period in days since the last rainstorm on fecal coliform densities measured in runoff samples, Homeland site.

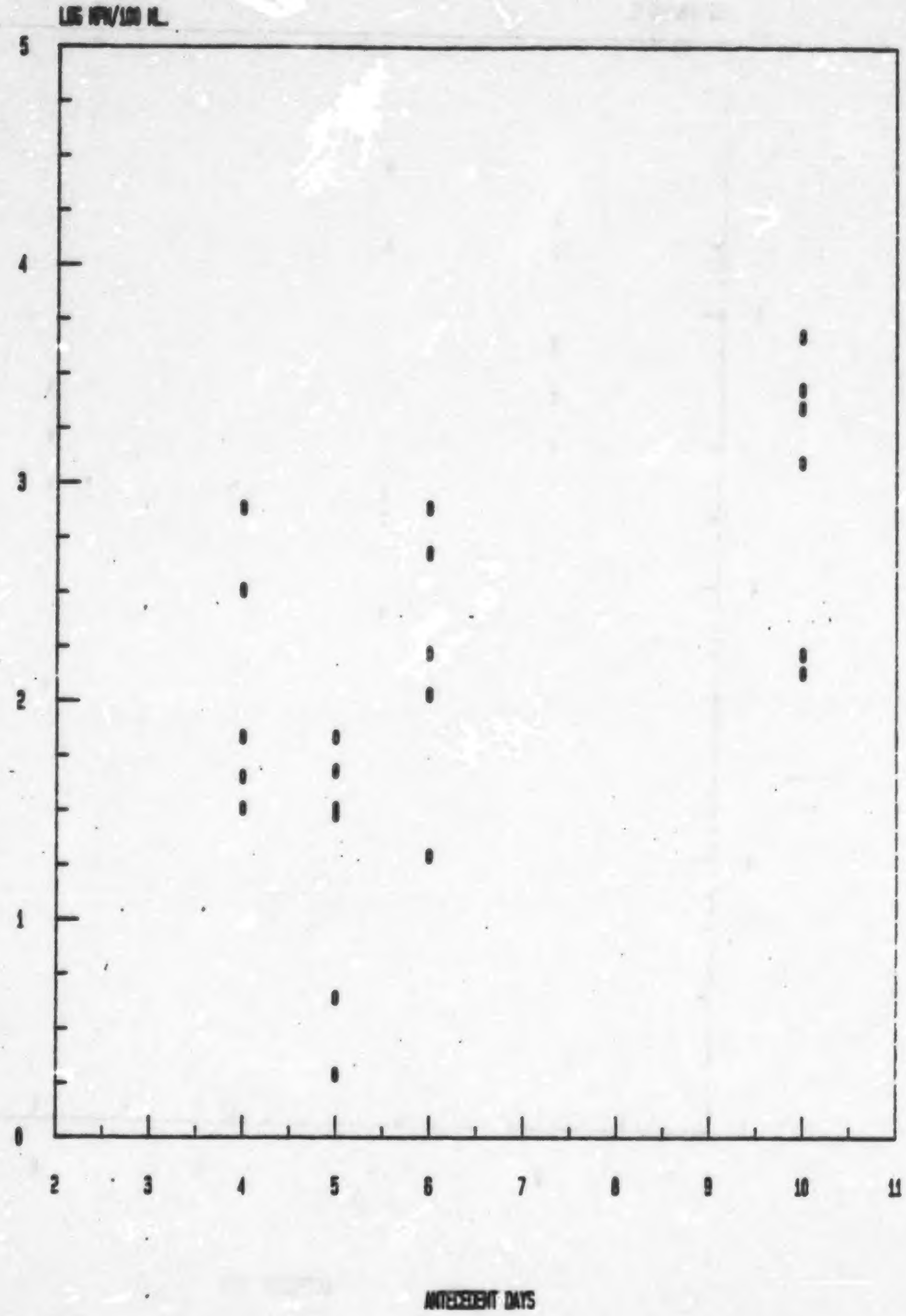

Figure 29. The effect of period in days since the last rainstorm on fecal coliform densities measured in runoff samples, Bolton Hill site.

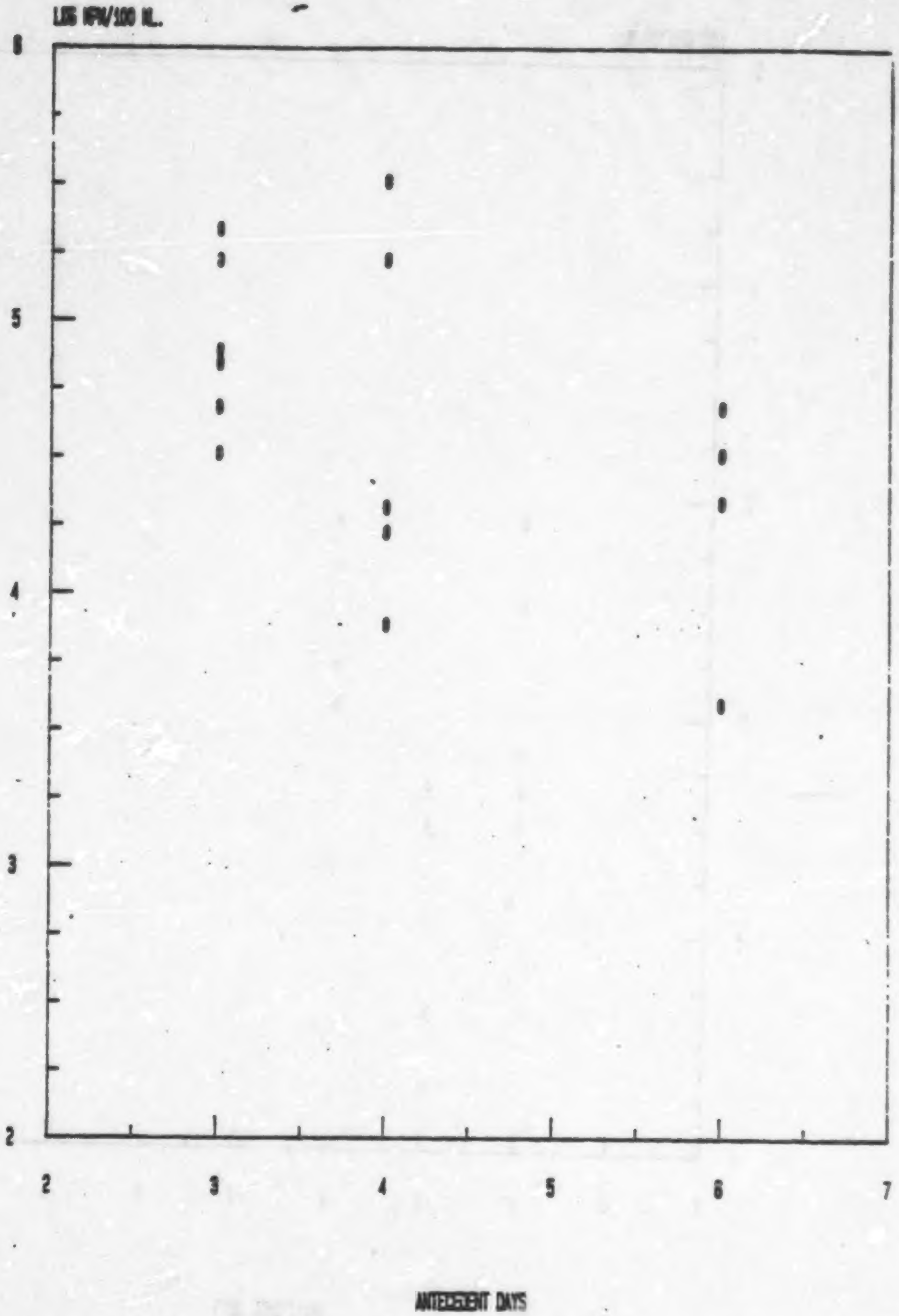

Figure 30. The effect of period in days since the last rainstorm on fecal coliform densities measured in runoff samples, Reservoir Hill site.

INDICATOR RATIOS

The relationship between the levels of fecal coliforms and fecal streptococci for runoff samples at each of the 4 sites is shown in Figures 31 to 34. Theoretical ratios are indicated by solid lines on each plot. The scatter of the data relative to the theoretical ratios can be seen. All FC/FS ratios for runoff samples from the Homeland site were less than 1.0. The frequency of samples from Reservoir Hill with FC/FS ratios greater than 4.0 was 75%, and only 19% of the samples had ratios less than 1.0. 44% of samples from the East Baltimore alley had ratios greater than 4.0 and 56% below 1.0

Figures 35 to 40 compare the levels of fecal coliforms and fecal streptococci for each source sample category. FC/FS ratios for all rat feces, street surface dust and dirt and refuse samples were below 1.0. 82% of the dog fecal samples had ratios less than 1.0 while 13% and 5% had ratios between 1.0 and 4.0 and greater than 4.0 respectively. The frequency of soil samples with FC/FS ratios of less than 1.0 was 94% and that for vegetation was 96%.

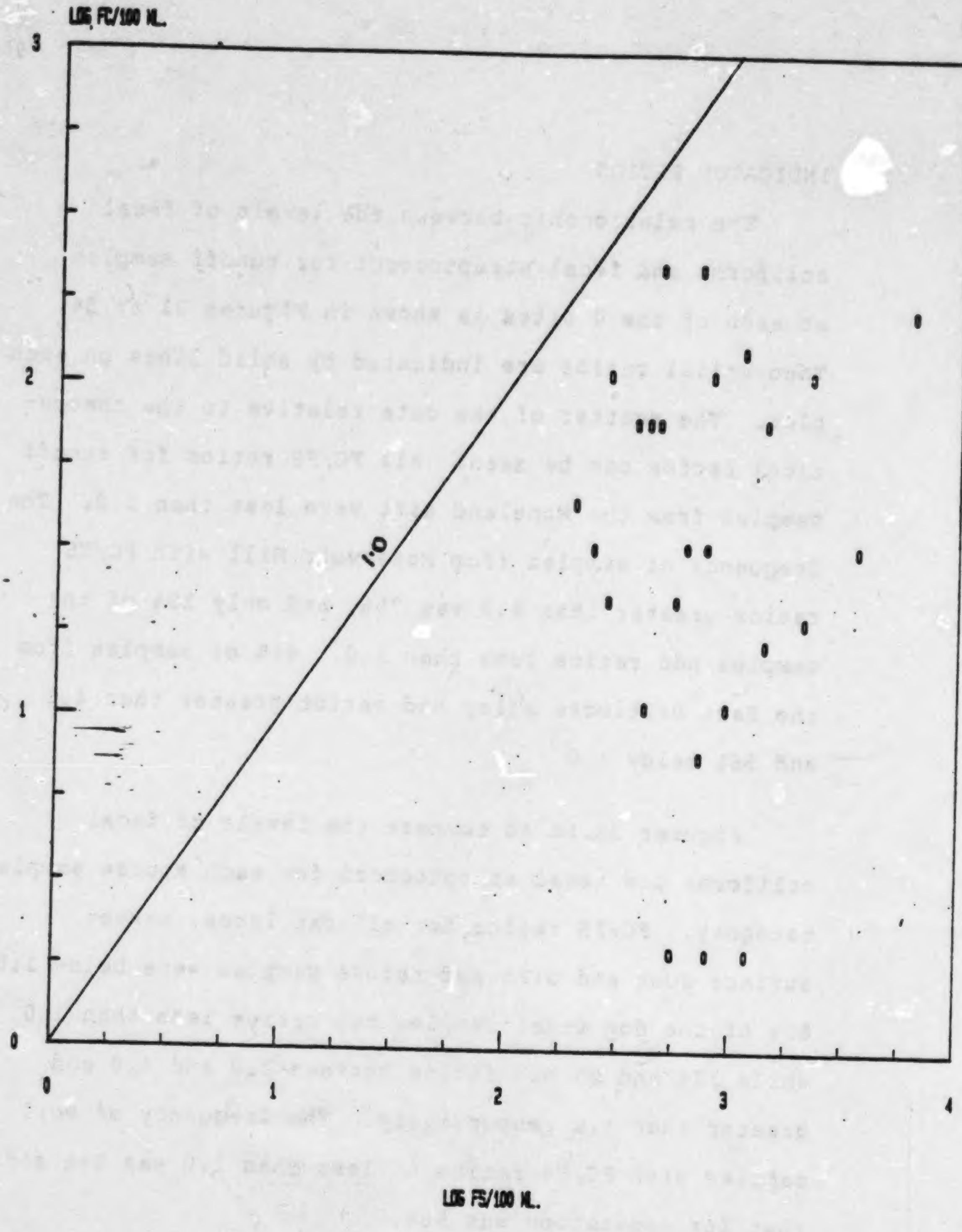

Figure 31. Ratio of fecal coliforms to fecal streptococci in runoff samples, Homeland site.

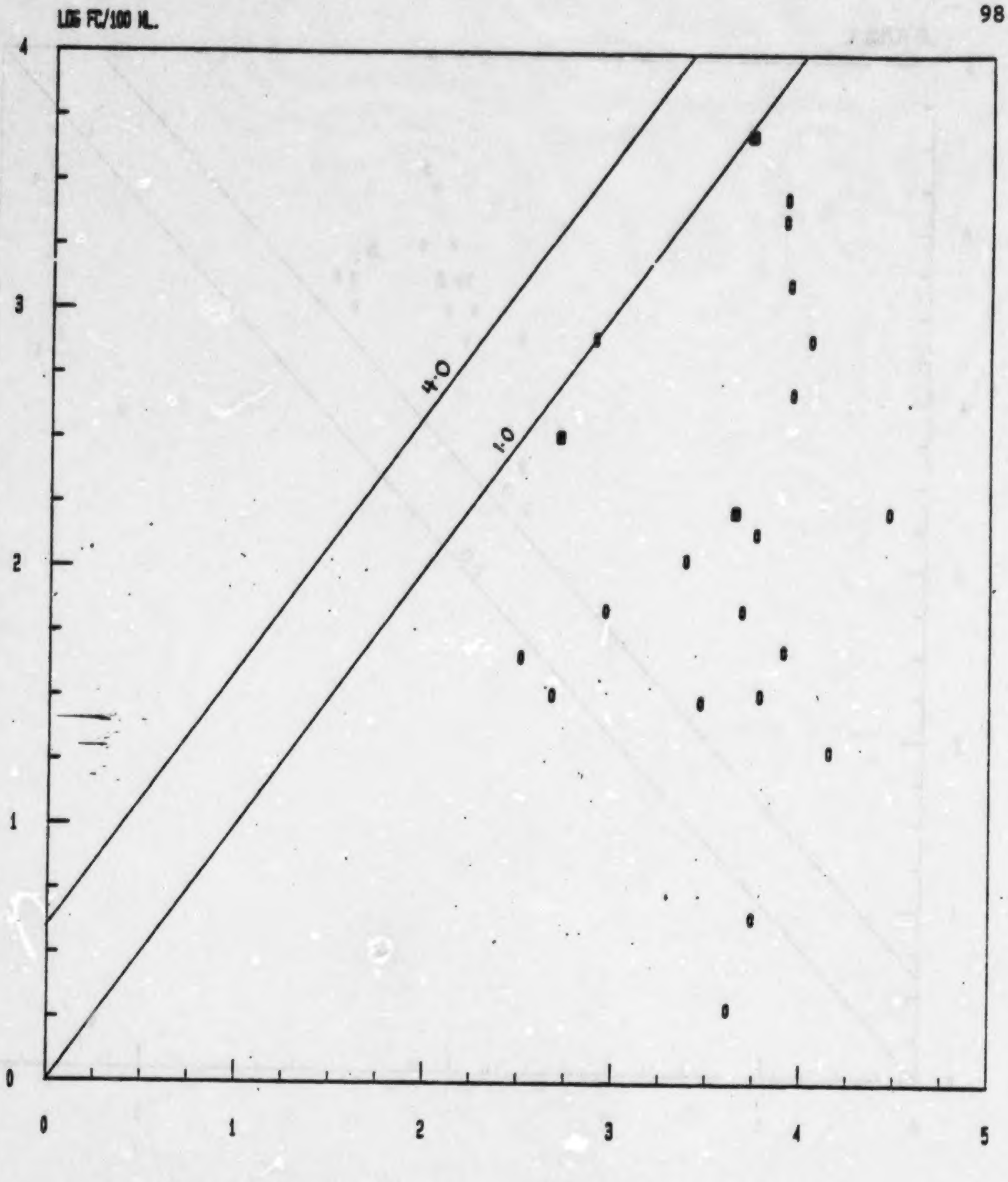

Figure 32. Ratio of fecal coliforms to fecal streptococci in runoff samples, Bolton Hill site.

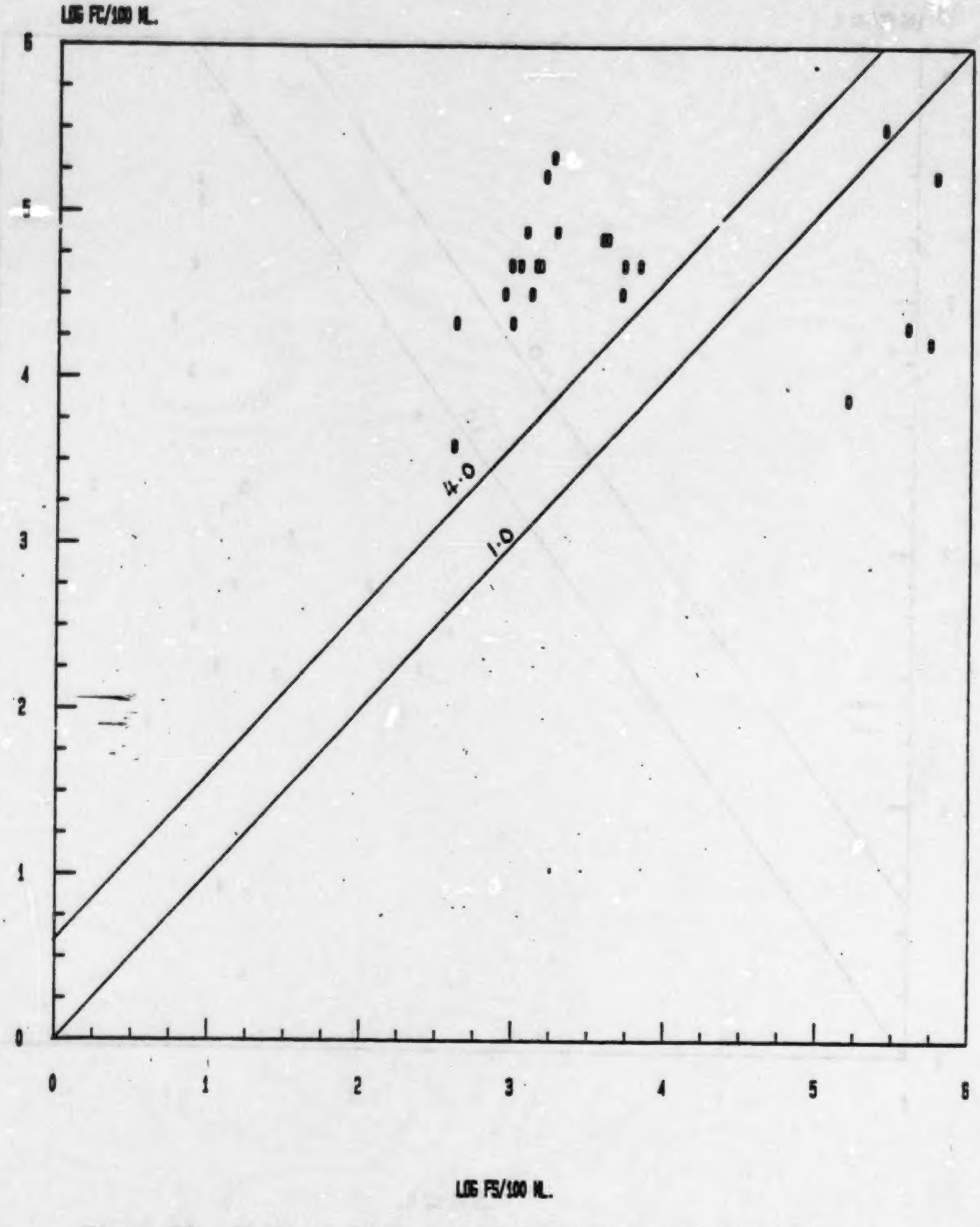

Figure 33. Ratio of fecal coliforms to fecal streptococci in runoff samples, Reservoir Hill site.

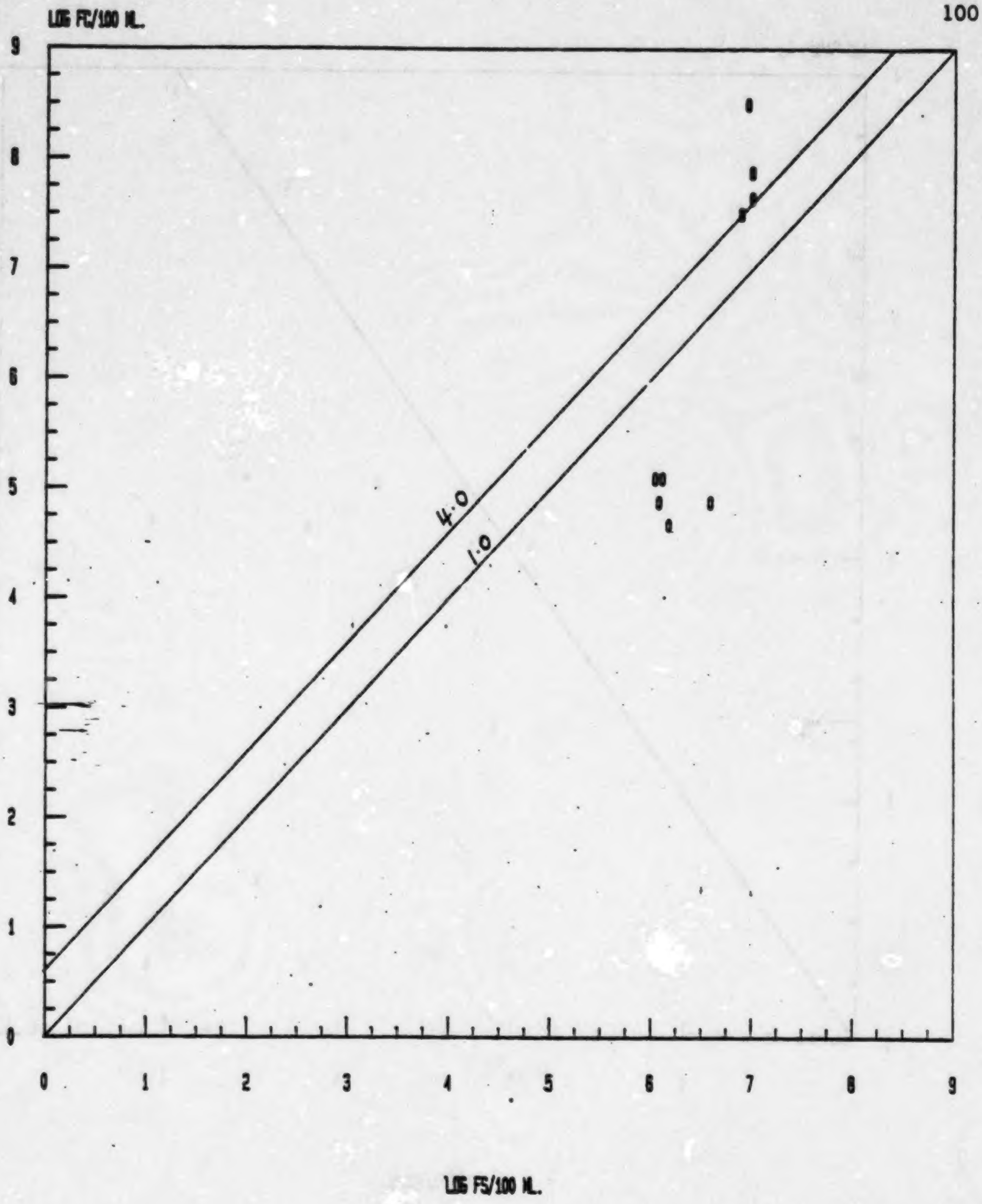

Figure 34. Ratio of fecal coliforms to fecal streptococci in runoff samples, East Baltimore site.

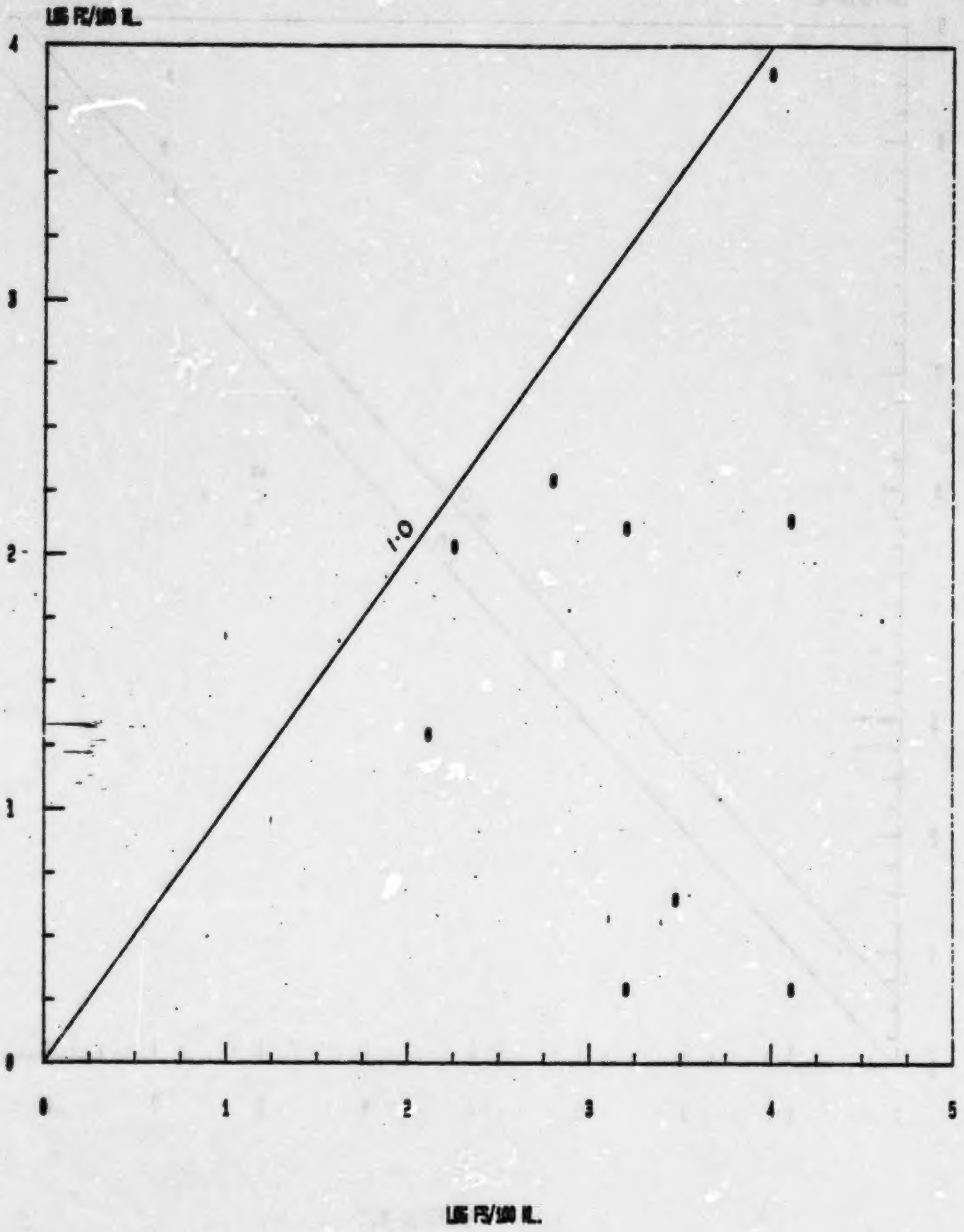

Figure 35. Ratio of fecal coliforms to fecal streptococci in street surface dust and dirt samples.

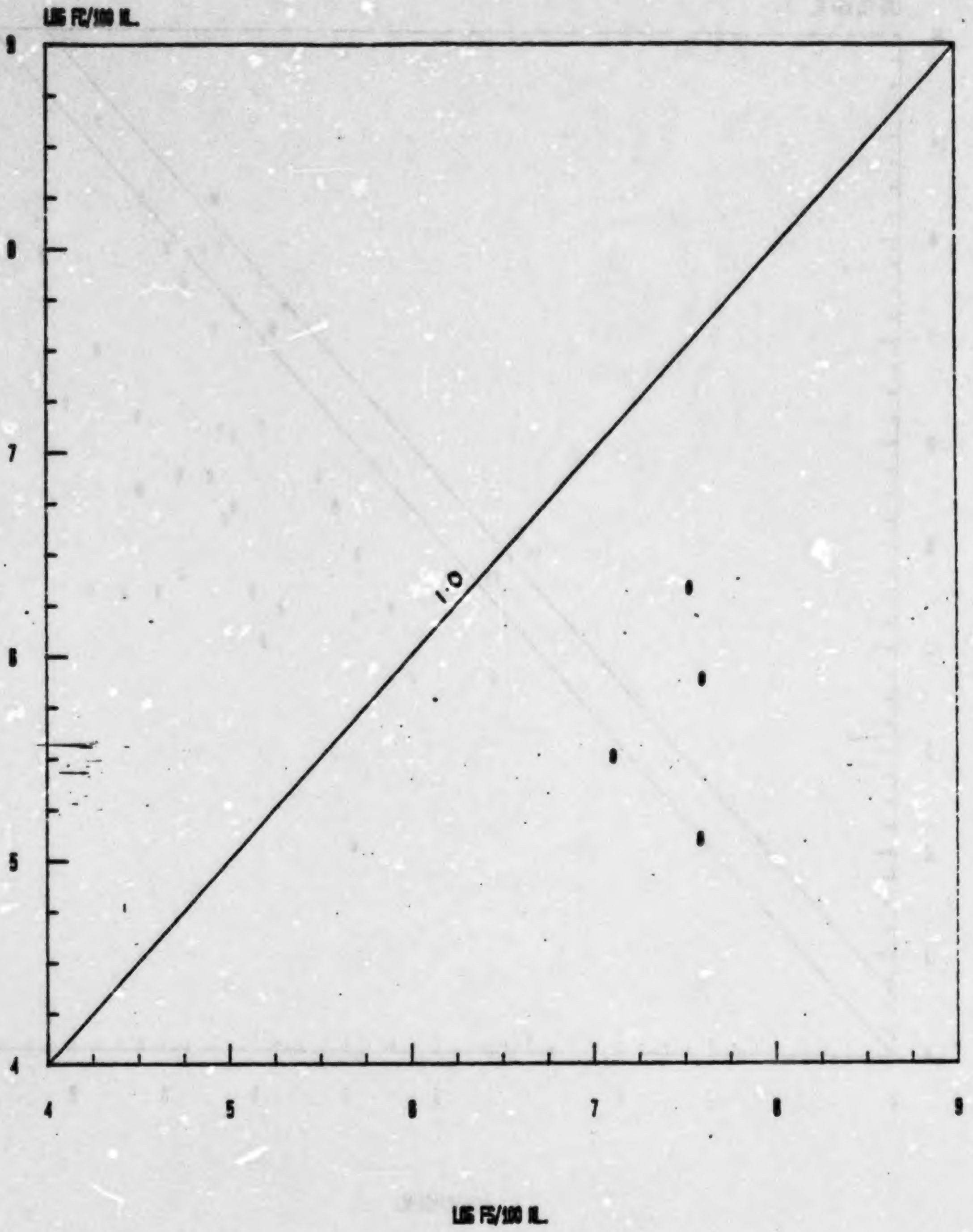

Figure 36. Ratio of fecal coliforms to fecal streptococci in rat fecal samples.

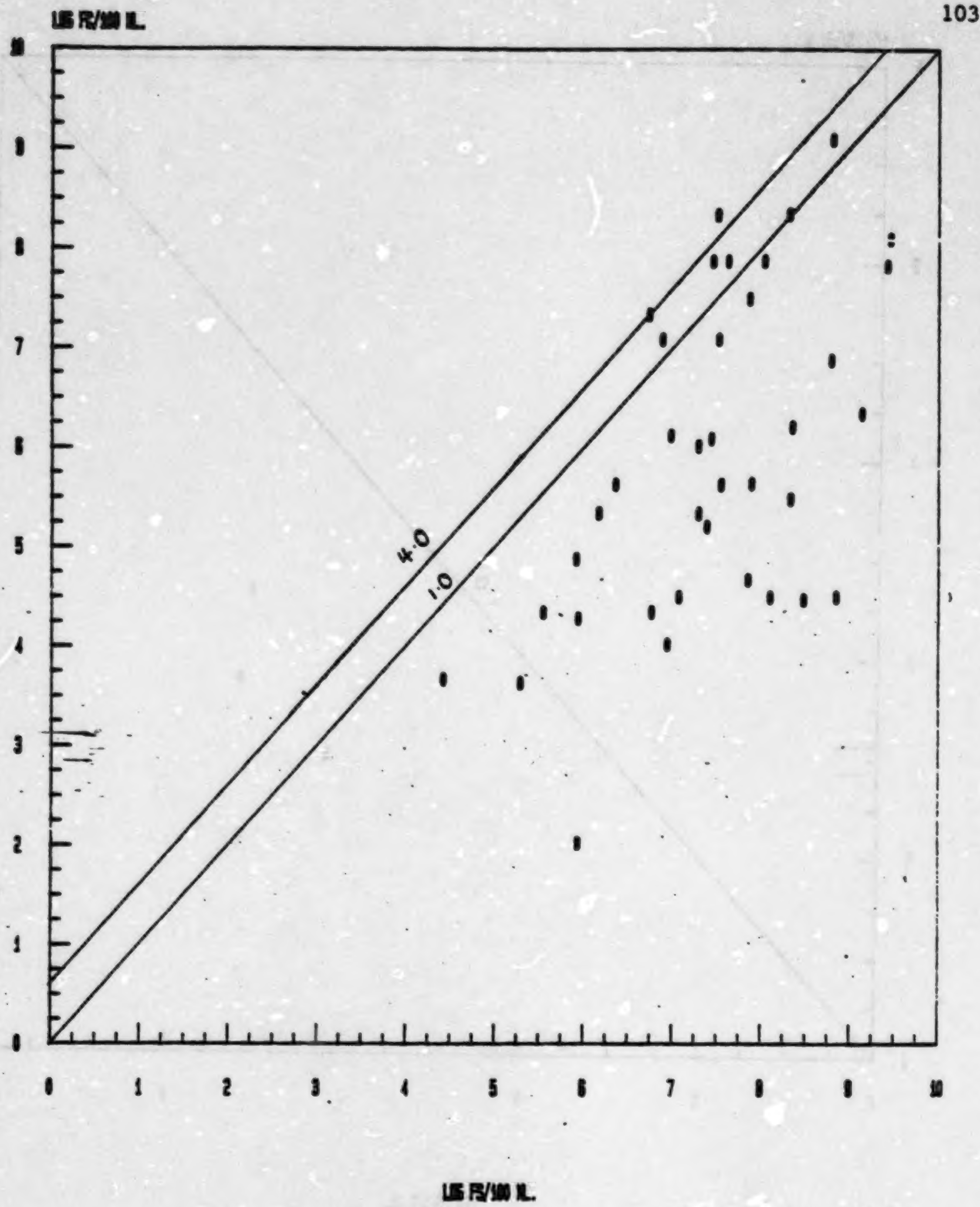

Figure 37. Ratio of fecal coliforms to fecal streptococci in dog fecal samples.

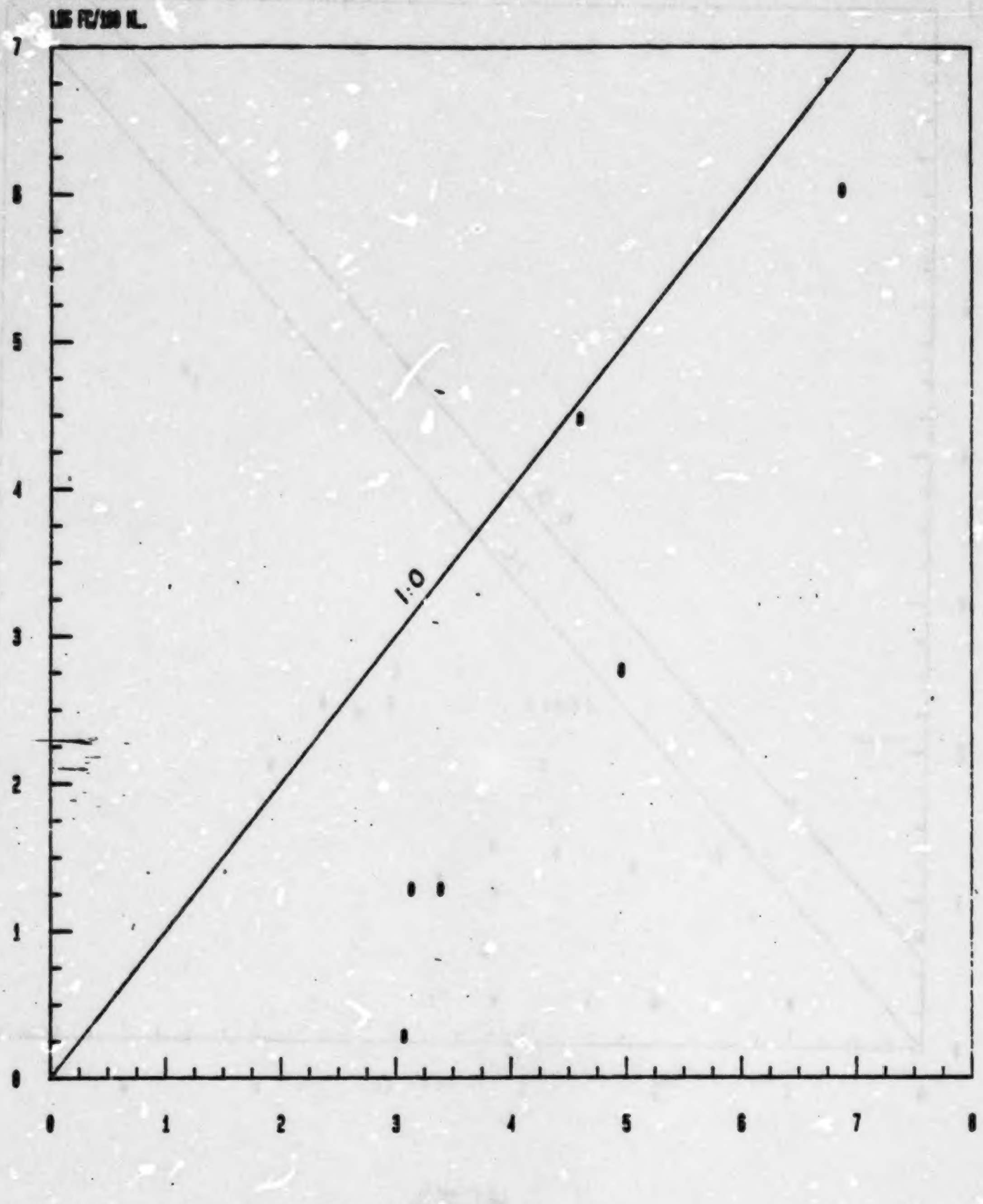

Figure 38. Ratio of fecal coliforms to fecal streptococci in uncovered refuse samples.

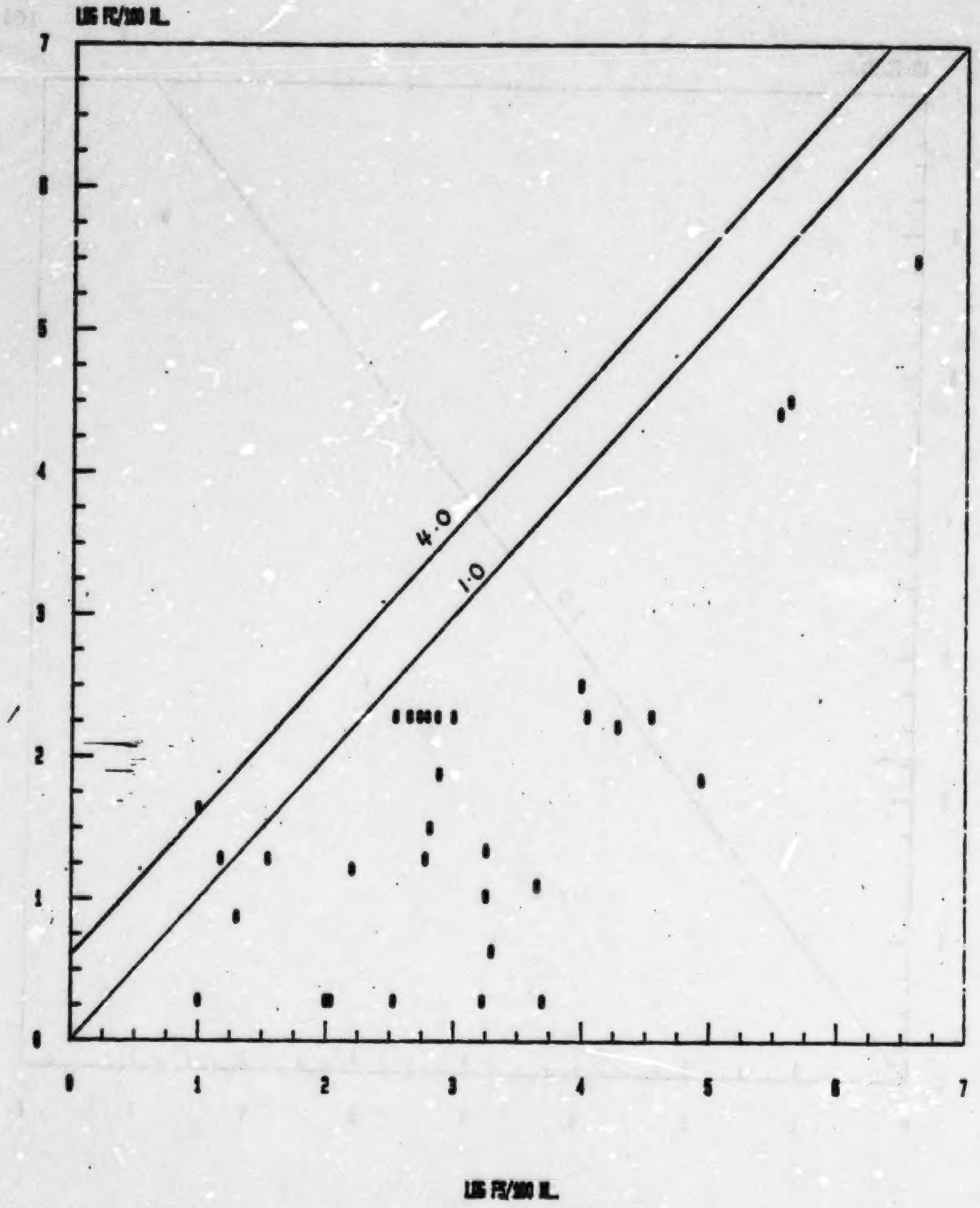

Figure 39. Ratio of fecal coliforms to fecal streptococci in soil samples.

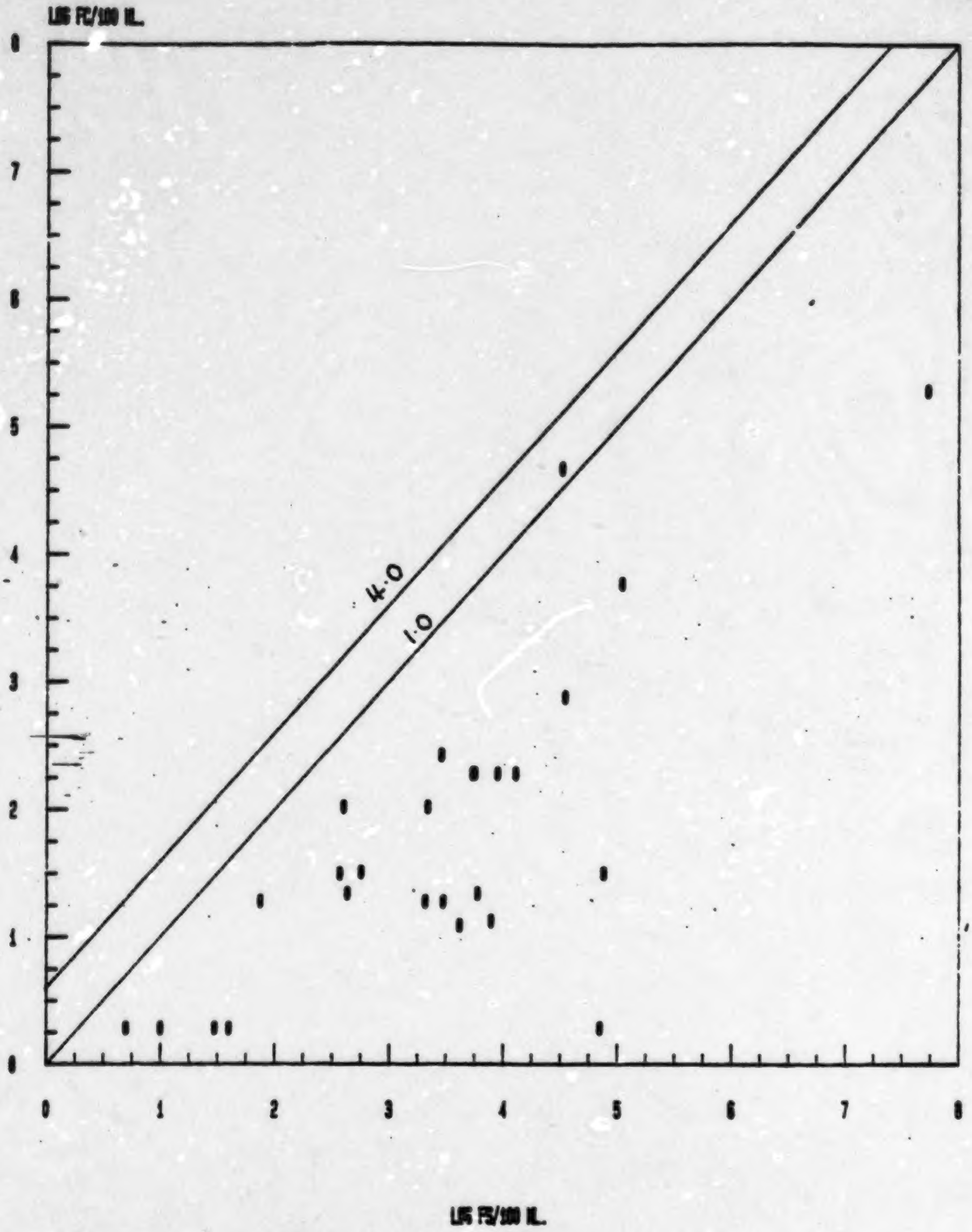

Figure 40. Ratio of fecal coliforms to fecal streptococci in vegetation samples.

DISCUSSION

INDICATOR LEVELS IN RUNOFF SAMPLES

It is evident from the tables and graphs presented in the Results section that there was considerable variation in the levels of each of the selected indicator organisms for runoff samples from the same alley location. Differences of a few orders of magnitude between the minimum and maximum densities for each microbial group were observed in samples from different runs at each loction. Smaller differences were observed between levels in samples from the same run. Variation ~~in the~~ microbial quality of storm runoff from a given area or even from a given storm is not uncommon and has been reported in previous stormwater studies (Olivieri, et al.,1977; Mallard, 1981). Betson and Buckingham (1969) observed differences of several orders of magnitude in indicator bacterial levels over a span of 2 minutes. Numerous factors will influence the levels of microorganisms in each water sample, and each "grab" sample reflects the microbial quality of the water at the time of collection. Of these factors, the wide ranges of bacterial indicator levels found in a variety of source inputs, as well as the spatial and temporal variations in source contributions probably play a significant part in the variation of microbial levels in the runoff.

Apart from variations within sites, it was also evident from the data presented that there were distinct differences in the microbial quality of runoff from the different alley locations. Runoff from the East Baltimore and Reservoir Hill sites which had higher population densities than the other 2 sites, and were generally in unsanitary condition, was significantly higher in microbial density than runoff from the Bolton Hill and Homeland sites. Although it had a moderately high population density, Bolton Hill was generally clean, and the Homeland site, which had a low population density, was also well-maintained. Of these latter two locations, Bolton Hill, which had more residents, had higher levels of microorganisms in runoff when compared to the Homeland site. The results thus strongly indicated that in urban areas with higher population densities, there was more contamination of the land environment due to the increased numbers of people and animals, and that the higher levels of contamination were reflected in the quality of the runoff.

Olivieri _et al_.(1977) in their stormwater study in Baltimore, also investigated the levels of microorganisms in background samples from raw sewage, urban streams in the area, and the Loch Raven reservoir, which is one of the city's sources for potable water. The reservoir and raw sewage samples provided information on the microbial water quality that can be expected under the best and worst conditons, respectively, for an urbanized area. A portion of their data was reproduced in Table 17, which shows the geometric mean indicator densities for Loch Raven reservoir samples and raw sewage.

Comparing the microbial levels from these samples to those from this study, it can be seen that the densities of indicator microorganisms in runoff from the 4 sites ranged from 1 to 5 orders of magnitude above those found in the Loch Raven reservoir. In the case of runoff samples from Reservoir Hill and the East Baltimore site, the levels of indicators approached the indicator densities of raw sewage. Olivieri et al.(1977) had demonstrated that storm water runoff is generally heavily contaminated and is of poor microbial quality. This study yielded data to show that runoff from areas that were densely populated as well as in poor sanitary condition was very heavily contaminated with indicator microorganisms, and, from a microbiological standpoint, was of much poorer quality than runoff from cleaner, less densely populated locations. There was no known contamination from sewer drains in any of the 4 sites investigated during the runoff events.

Table 17. GEOMETRIC MEAN DENSITIES OF INDICATOR MICROORGANISMS IN RESERVOIR AND RAW SEWAGE SAMPLES

Reference: Olivieri et al., 1977

Sample	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml	Fecal Strep No./100ml
Loch Raven reservoir water	2.6E1	1.5E1	1.0E1
Raw sewage	2.3E7	6.3E6	1.2E6

INDICATOR LEVELS IN SOURCE SAMPLES

Wide variations were also found in the levels of indicator bacteria in each of the source sample categories and to a lesser, but still considerable extent, in indicator levels in each category from each individual site. This was not unexpected, as even similar types of samples from the same location can be contaminated in numerous different ways. In the case of fecal material, refuse, and street surface dust and dirt, different samples can be deposited under varying conditions and from numerous sources.

Comparison of mean indicator densities from the different sample categories clearly demonstrated that animal feces contained the highest levels of indicator microorganisms compared to the other source sample categories, and thus has the highest potential of contributing to the microbial pollution of runoff waters. High mean densities of bacteria were found in animal fecal material, independent of the site where it was collected. Hence, in considering the potential microbial contribution from this source for different sites, it might be useful to estimate the magnitude of fecal material deposited, and use such an estimation as an index of pollution.

One of the most common estimators of dog population density assumes that dogs to humans are in the ratio of 1:7, based on the surveys of Marx and Furcolow (1969). This ratio, which is equivalent to one dog for 2.36 housing units or 42.4% ownership, was found to be consistent with Beck's (1972) interview estimates of dog ownership in Baltimore. Beck further estimated that with 30 to 50% dog ownership and several families per building, nearly every backyard which borders the alleys in the inner city contains at least one captive dog.

In order to make an estimation of the amount of the fecal material deposited by dogs in the 4 sites investigated in this study, a ratio of 1 dog to 1 backyard bordering the alleys was assumed, regardless of the number of household units in each building. The Bureau of Preventable Diseases estimates a mean fecal output of 0.34kg/day/dog (0.75lb/day/dog), based on studies of 482,829 large dogs (Beck,1972). The total fecal mass possible in each alley is thus the product of this mean output and the number of dogs. As shown in Table 18, multiplying the total mass in grams by the mean MPN/gram for each site gives an estimate of the potential microbial load from dog feces deposited daily. Note that the total fecal mass is a conservative estimate as it does not take into account stray

Table 18. POTENTIAL MICROBIAL CONTRIBUTION (INDICATOR MICROORGANISMS) FROM DOG FECES

			Potential Microbial Load		
Site	No. of dogs (= No. of backyards)	Total fecal mass grams/day	Total Coliform MPN/day	Fecal Coliform MPN/day	Fecal Strep No./day
Homeland	5	1.7E3	1.9E10	1.6E9	2.7E10
Bolton Hill	20	6.8E3	4.1E9	3.0E9	3.4E10
Reservoir Hill	14	4.8E3	9.6E9	6.7E9	9.1E11
East Baltimore	10	3.4E3	1.5E10	4.4E9	1.6E11

dogs that wander through the alleys or dogs belonging to non-residents that are either allowed to run loose or are walked by their owners in the area. Stray dogs are also more numerous in poorer areas where garbage piles provide them with a source of food, and where the residents either regard them with more sympathy or are less aware of city services for pick-up of strays (Beck, 1972).

As can be seen from Table 18, the potential microbial contribution from dog feces was extremely high regardless of site. Not all of the fecal material deposited on the ground always gets washed off by runoff from a single storm. The amount of feces that eventually gets incorporated into runoff would depend on factors such as the duration and intensity of the rainfall, the particle size and transport capacity of the waste material, as well as the location in the drainage area where defecation occurs. Nevertheless, the potential for microbial pollution from this source was very great.

Table 19 gives the mean bacterial loads per "rain" event of 1.5 hour duration in runoff from the different sites, which are an extension of the calculations for Table 16. The microbial loads in runoff from each of the 4 sites were within 3 orders of magnitude difference

Table 19. ESTIMATED MEAN MICROBIAL LOADS PER RAIN EVENT (1.5 HR DURATION) IN RUNOFF SAMPLES

Site	Total Coliform MPN/rain event	Fecal Coliform MPN/rain event	Fecal Strep No./rain event
Homeland	1.3E7	2.1E6	3.1E7
Bolton Hill	4.9E7	2.3E6	1.1E8
Reservoir Hill	8.1E9	4.0E9	8.1E9
East Baltimore	4.6E12	2.0E12	2.1E11

when compared to the corresponding estimated fecal loads that were possible at each site (Table 18). The results obtained strongly suggest that a large proportion of microorganisms in runoff could come from the feces of animals, particularly pets, in the drainage areas.

Rat feces was harder to locate in the study sites due to the smaller size of the droppings, and as a consequence, fewer samples were examined. Nevertheless, the presence of these rodents in the poorer dirtier neighborhoods was very obvious, as they were observed on several occasions in these alleys. Beck (1972) has noted that excessive accumulations of garbage in these neighborhoods attract a large number of dogs as well as rats. In addition, dogs supply food for rats by disrupting and overturning garbage, and there are instances where rats use dog feces for food and moisture. Hence, a densely populated area with large numbers of dogs and unsanitary conditions such as in East Baltimore and Reservoir Hill would be expected to have a high rat density.

The rat fecal samples examined in this study had levels of indicator organisms as high as those found in dog feces. Since there are probably large populations of rats in poorly maintained neighborhoods, there is also a high possibility of their fecal material forming a major source of microorganisms in runoff waters.

Samples of uncovered refuse were only collected from the East Baltimore and Reservoir Hill sites as garbage from the other 2 sites was always enclosed in trash bags and placed in covered trash cans. This sample category is an often overlooked source of microorganisms. In the more affluent, well-maintained neighborhoods like Homeland and Bolton Hill, microbial pollution from refuse does not pose a serious problem. However, the massive piles of garbage often found in the poorer areas such as East Baltimore and Reservoir Hill not only attract dogs and rats (which, as already discussed, are themselves prime contributors to microbial pollution) but also harbor high densities of microorganisms that could potentially be washed off by rainfall.

Beck (1972) attributed the excessive amounts of garbage in the less affluent Baltimore neighborhoods to reasons such as high population density; only 2 weekly pick-ups of refuse regardless of density; no provisions for preventing trash cans from being knocked over by dogs; inadequate number of cans; lack of proper motivation to police alleys or enforce litter codes as residents are not the land owners; and lack of garbage disposal units in high density areas, so that trash is often just left along the alleyways. Even where refuse was collected, there was often ample litter left on the ground as can be seen from Figures 41a and 41b which show the same site at Reservoir Hill immediately before and after trash collection by a city collection crew.

Levels of indicator organisms in street surface dust and dirt, soil and vegetation samples were relatively low compared to animal feces and refuse samples. Of the study sites, Homeland, which had the lowest population density, also had the lowest indicator levels for samples in all 3 categories, generally 10-fold lower than levels for Bolton Hill and Reservoir Hill samples, and several orders of magnitude lower than those for the East Baltimore alley. It would appear that contamination from these sources is related to the potential for contamination from human and animal populations in the area. However such an assumption should be treated with caution as soil and vegetation are known reservoirs for large numbers of nonfecal coliforms which are relatively ubiquituous in nature (Van Donsel _et al_.,1967; Geldrich,1968). Except for the East Baltimore alley, fecal coliform levels in street surface dust and dirt, soil and vegetation samples were relatively low. It would be difficult to estimate the extent to which these 3 types of surfaces receive contamination from human and animal sources in the area.

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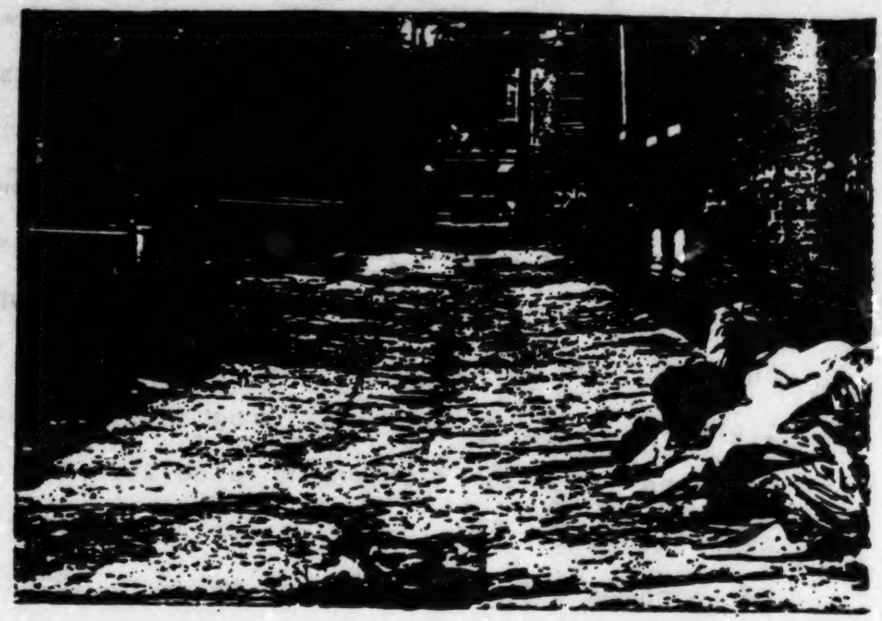
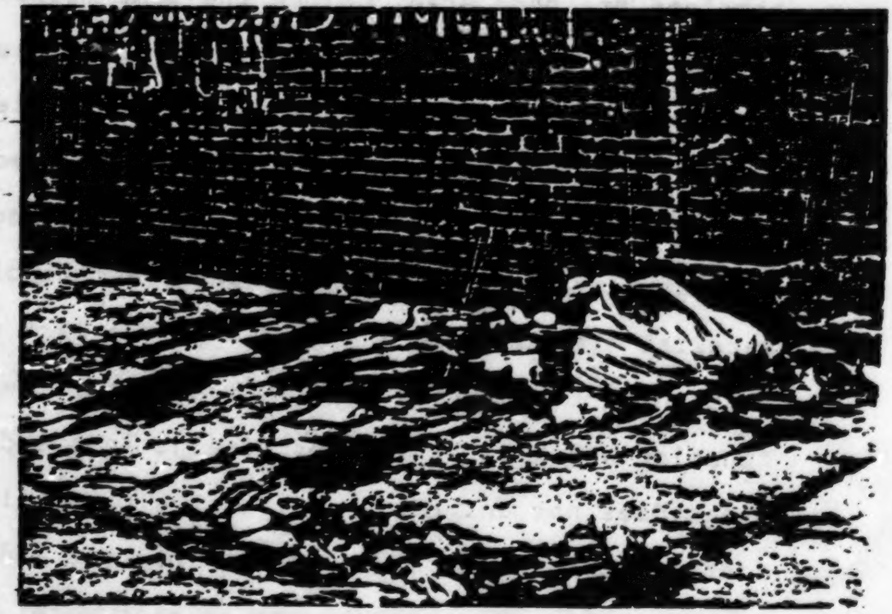


41a. Before refuse collection



41b. After refuse collection

Figure 41. Inefficiency of refuse collection process

SALMONELLA

Salmonella was not detected in any of the runoff samples from the study sites at the lower sensitive limit of the assay. The earlier stormwater study in Baltimore (Olivieri et al.,1977) had found low levels of the pathogen, with geometric mean densities ranging from 5.7 to 30/10 liters in runoff at sites where there were no known sewage overflows. Recent investigations in this laboratory (in progress) have so far revealed the results shown in Table 20. The samples examined were storm runoff composites for each entire homogeneous watershed. As can be seen, levels of Salmonella enumerated are also low, particularly for the Homeland drainage area. Since the same methodology was employed in all these investigations, one reason for the positive detection of Salmonella in the samples from the larger drainage areas may be the increased chance of contamination from any source of the pathogen, since a larger area and presumably greater amount of source material are considered. Secondly, the lack of knowledge concerning the existence of known sanitary sewer malfunction and leaks into storm drains in the drainage areas does not entirely rule out the possibility of contamination from this source. This was indicated by the detection of enteroviruses in stormwater from sites where there were no known sanitary sewer inputs (Olivieri et al.,1977). Poor maintenance and infrequent monitoring of the sewer system for leaks may result in such deficiencies being overlooked.

Table 20. DENSITIES OF SALMONELLA IN COMPOSITE STORM RUNOFF SAMPLES

(Personal communication, V.P. Olivieri, Johns Hopkins Univ.)

Site	Date sample collected	MPN Salmonella/10 liters
Homeland	7/21/82	1.2E0
Homeland	7/28/82	<3.0E1
Homeland	8/2/82	8.9E0
Homeland	8/12/82	<1.2E0
Homeland	8/25/82	<6.6E0
Bolton Hill	6/7/82	2.2E0
Bolton Hill	8/2/82	4.7E2

Of the source samples examined for Salmonella, only dog feces yielded positive results. Table 21 compares the frequency of detection of this pathogen in this study with those obtained from other studies. Most of these studies were qualitative investigations on freshly voided feces. Despite the comparatively small number of samples examined in this study, the results obtained did not seem incompatible with the range of prevalence rates covered by other studies. It was not possible to determine the degree of freshness of fecal samples collected from the Baltimore alleyways and backyards, apart from a qualitative assessment of the moisture content, but it was observed that samples that were positive for the pathogen were in varying states of dryness when collected.

With a geometric mean density of 1.6/gram and a detection frequency of 19%, the estimated microbial load for this pathogen from a site with 20 dogs (refer Table 16) would be in the order of 2100 Salmonella/day. For a rain event of 1.5 hours duration and a flow of 0.6 liters/second (average of all the trials where flow was measured), the total runoff volume generated would be approximately 3,500 liters. If half the estimated Salmonella load were to be washed off during the course of this event, the estimated MPN for Salmonella should then be about 1100/3500 liters or 3/10 liters.

Table 21. COMPARISON OF THE FREQUENCY OF DETECTION OF SALMONELLA IN DOG FECES

Report	Total no. of samples	No. positive for *Salmonella*	% positive
This study	32	6	19.0
Wolff *et al.* (1948)	100	18	18.0
Watt and Decapito (1950)	1156	39	3.4
Galton *et al.* (1952)	8157	2252	27.6
Stewart and Decapito (1953)	705	36	5.1
Butler (1965)	29	7	24.0

There may be several reasons why this does not show up in the assays of the runoff samples. One is that the number of Salmonella organisms that eventually get washed off by rain are so small that, on dilution in the runoff, the levels are too low to be detected by the assay employed. Alternatively, the actual mean densities of Salmonella in most dog feces may be much lower than the geometric mean found for this study, which was obtained from only 6 positive samples, so that the resulting load might be much lower.

Barring the possibility of contamination from an external source, soil and vegetation did not appear to constitute any appreciable reservoirs for Salmonella organisms. Cherry et al. (1975) detected Salmonella in wet samples of vegetation collected from an urban stream, but obtained negative results for dry vegetation samples and soil samples. Thomason et al. (1975) also found that the moisture content of the sample had a significant effect on Salmonella detection in soil and vegetation. All positive samples were collected in or near standing or running water. The possibility of contamination from this contact cannot be ignored, especially since animals can be expected to congregate for water. Recent investigations in this laboratory have also come up with negative results at the lower sensitive limit of the assay for 12 soil and 4 vegetation samples from a Baltimore County landfill (personal communication, V.P.Olivieri, Johns Hopkins University).

FC/FS RATIOS

The FC/FS ratio has been employed frequently to determine whether the source of microbial contamination was of human or animal origin (Geldrich and Kenner,1969; Geldrich,1972; Feachem,1974; Skinner et al.,1974). Fecal streptococci have been found to be present in greater numbers than coliform bacteria in animal feces (Cooper and Ramadan,1955; Bartley and Slanetz,1960; Kenner et al.,1960; Mundt,1963; Rogers and Sarles,1964; Geldrich,1968). In human feces, however, fecal coliforms were found in greater numbers than fecal streptococci. Geldrich and Kenner (1969) reported that FC/FS ratios for human feces were greater than 4.0, and for animal feces, separate stormwater systems and farm drainage were less than 0.7. The ratios between 0.7 and 4.0 were difficult to interpret. The limitations with regard to the application of the FC/FS ratio have been discussed in the Literature Review section.

Table 22 shows the frequency of occurrence of FC/FS ratios in runoff samples from the 4 alleyways. All of

Table 22. FREQUENCY OF OCCURRENCE OF THE FC/FS RATIO IN RUNOFF SAMPLES

	Ratio FC/FS		
Site	Less than 1.0	1.0 to 4.0	Greater than 4.0
Homeland	26	0	0
Bolton Hill	25	1	0
Reservoir Hill	5	1	18
East Baltimore	5	0	4

the Homeland samples had ratios below 1.0 and this was true for all except one of the Bolton Hill samples. However, the frequency of samples from Reservoir Hill with FC/FS ratios above 4.0 was 75%, and only 19% (5 of 6 samples from a single trial) had ratios less than 1.0. The East Baltimore alley had 44% samples with ratios greater than 4.0 and 56% below 1.0, the 2 groups representing 2 separate sets of samples from 2 individual trials.

Table 23 shows the frequency of ocurrence of FC/FS ratios in the 6 categories of source samples. FC/FS ratios in all rat feces, street surface dust and dirt and refuse samples were less than 1.0. Of the dog fecal samples, 5% had ratios greater than 4.0, 13% had ratios between 1.0 and 4.0 and 82% less than 1.0. 94% of soil samples had FC/FS ratios below 1.0 as did 96% of the vegetation samples.

It was not surprising to find some samples of animal feces with FC/FS ratios greater than Geldrich and Kenner's 0.7 ratio, as most of the samples collected from the alleys were not freshly voided as would be the case with samples from laboratory animals. Time growth and differential die-away of the 2 groups of microorganisms would alter the initial ratios vastly. It was impossible to determine when the fecal samples were deposited prior to collection.

Table 23. FREQUENCY OF OCCURRENCE OF THE FC/FS RATIO IN SOURCE SAMPLES

	Ratio FC/FS		
Sample	Less than 1.0	1.0 to 4.0	Greater than 4.0
Street surface dust and dirt	9	0	0
Dog feces	31	5	2
Rat feces	4	0	0
Soil	31	1	1
Vegetation	27	1	0
Uncovered refuse	6	0	0

The frequency of occurrence of FC/FS ratios in the source samples did not entirely explain the frequency pattern for runoff samples from Reservoir Hill and East Baltimore, with the large percentages of samples having ratios above 4.0. Personal interviews with some residents in the houses bordering the alleys and careful observation did not reveal any evidence of leaks from sewer pipes or drains in the area. Discarded diapers had been observed on a few occasions in garbage piles in alleys in the 2 neighborhoods, but not in the selected alleys during the experimental trials. Further subtle questioning did not reveal any other knowledge of direct human fecal contamination. In this light, while acknowledging that such contamination might still be possible without being immediately obvious, it is suggested that further doubt should be cast on the applicability of FC/FS ratios to storm runoff studies in urban areas where a multitude of non-point sources may contribute to microbial pollution. Geldrich and Kenner (1969) had stressed that the FC/FS ratios are most useful when the microbiological density data are collected near the origin of the sources. However, further limitations are imposed when the microbial levels in the potential sources of contamination are also subject to alteration by environmental factors and differential die-off even before incorporation into runoff waters.

CONCLUSIONS

1. High densities of indicator microorganisms were found in urban sites that were generally in poor sanitary condition and had high population densities.
2. Densities of indicator microorganisms in runoff from sites that were kept in clean condition were lower than densities in runoff from dirty sites, and higher densities were obtained from the more densely populated clean site as opposed to the less densely populated clean site.
3. The densities of indicator organisms in runoff did not show any marked correlation with flow or antecedent rainfall.
4. High densities of indicator microorganisms were found in animal feces, which may form a large reservoir for potential microbial pollution of runoff waters.
5. Next to animal feces, uncovered refuse was found to be a potential contributor of large numbers of microorganisms to runoff. Street surface dust and dirt, soil and vegetation are also potential sources, but to a lesser extent in terms of microbial numbers.
6. Low levels of Salmonella were detected in dog feces, but recovery was negative for runoff samples. The

extent to which the potential load of Salmonella from dog feces could be incorporated into runoff was not clear.

7. Salmonella was not detected in any of the street surface dust and dirt, soil or vegetation samples examined. These materials probably do not harbor the pathogen in any appreciable number.
8. The applicability of the FC/FS ratio to the determination of the sources of pollution in urban runoff should be seriously questioned, considering the multitude of non-point sources and environmental factors that are involved.

RECOMMENDATIONS

1. Microbial density data alone cannot be sufficient to form a basis upon which policy control measures regarding urban runoff pollution are determined. Apart from quantitative measurements of bacterial densities and statistical manipulation of the numbers obtained, a qualitative field survey for potential sources of pollution should be recommended. Unfortunately, it is often omitted. Differences in the general sanitary conditions of the sites investigated in this study were immediately obvious even upon casual observation. It is highly probable, judging from the results of this study, that the microbial quality of runoff from "dirty" sites would improve significantly if more attention were paid to upgrading sanitation and stricter enforcement of litter laws in these areas. Such measures may be more cost-effective than attempts to disinfect large volumes of storm runoff.
2. Animal feces in urban areas pose an aesthetic problem as well as the question of public health, as the feces constitute a reservoir for human pathogens. Dogs, especially, abound in urban areas because of their popularity as domestic pets and as watch dogs. Because of their size, their fecal wastes are

greater in magnitude than those of other common urban pets and pests. In addition, there is in most cities a sizeable population of free-ranging dogs, whose contribution to microbial pollution of runoff would not be affected by the institution of "scoop laws". Dog control would have to be exercised more strictly if control measures aimed at this pollution source are to be effective.

3. Additional data on hydrologic processes affecting non-point pollution from surface materials in urban areas may be useful to help determine the extent to which indicator microoganisms residing in source materials are eventually incorporated into runoff waters.
4. Information on the survival of natural bacterial populations would also be useful to better evaluate the impact of accumulation of source material on urban runoff waters, and the impact of the storm runoff on receiving bodies of water.

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APPENDIX A. LEVELS OF BACTERIA, RUNOFF SAMPLES.

RUN	STATION	SAMPLE	DATE	TIME, MINS.	FLOW, L/SEC	MPN TC	MPN FC	MPN FS
1	EB	1	110681	15	NM	490000000	330000000	9100000
1	EB	2	110681	30	NM	490000000	79000000	10000000
1	EB	3	110681	45	NM	110000000	46000000	10000000
1	EB	4	110681	60	NM	33000000	33000000	7800000
1	EB	C	110681	NM	NM	330000000	70000000	9200000
2	EB	1	110981	5	NM	330000	130000	1300000
2	EB	2	110981	25	NM	130000	79000	3900000
2	EB	3	110981	45	NM	490000	79000	1200000
2	EB	4	110981	65	NM	280000	49000	1500000
2	EB	5	110981	85	NM	230000	130000	1300000
2	EB	C	110981	NM	NM	230000	130000	1100000
3	BH	1	111281	5	NM	68	20	14000
3	BH	2	111281	20	NM	1700	170	29000
3	BH	3	111281	35	NM	2200	790	11000
3	BH	4	111281	50	NM	3300	490	8800
3	BH	5	111281	65	NM	1100	170	4500
3	BH	6	111281	80	NM	3300	170	4400
3	BH	7	111281	95	NM	340	110	2400
3	BH	C	111281	NM	NM	1100	210	10000
4	RH	1	112481	5	0.2745	23000	7800	160000
4	BH	2	112481	20	1.4575	46000	17000	550000
4	RH	3	112481	35	1.4575	330000	170000	600000
4	RH	4	112481	50	1.4575	700000	170000	590000
4	RH	5	112481	65	1.4575	110000	21000	390000
4	RH	6	112481	80	1.4575	330000	330000	270000
4	RH	C	112481	NM	NM	330000	170000	280000
5	BH	1	120181	5	0.0481	460	230	28000
5	BH	2	120181	20	0.0764	140	45	25000
5	BH	3	120181	35	0.0764	330	45	22000
5	BH	4	120181	50	0.1132	1600	61	27000
5	BH	5	120181	65	0.1132	6100	78	27000
5	BH	6	120181	80	0.1132	1400	45	20000
5	BH	C	120181	NM	NM	310	45	25000
6	HO	1	121181	10	0.1585	360	45	200
6	HO	2	121181	25	0.2123	330	20	2100
6	HO	3	121181	40	0.3453	1100	110	2200
6	HO	4	121181	55	0.3453	490	2	1260
6	HO	5	121181	70	0.3453	280	17	1400
6	HO	6	121181	85	0.3453	56	2	800
6	HO	C	121181	NM	NM	28	14	1400
7	BH	1	22682	5	0.4245	1100	33	6000
7	BH	2	22682	20	0.5971	2200	49	8000
7	BH	3	22682	35	0.6962	2300	70	4800
7	BH	4	22682	50	0.6962	490	31	2900
7	BH	5	22682	65	0.6962	79	5	5500
7	BH	6	22682	80	0.6962	27	2	4100
7	BH	C	22682	NM	NM	790	11	5100

RUN	STATION	SAMPLE	DATE	TIME,MINS.	FLOW,L/SEC	MPN TC	MPN FC	MPN FS
8	HO	1	32382	5	0.5066	330	11	410
8	HO	2	32382	20	0.5971	140	23	560
8	HO	3	32382	35	0.6962	170	33	610
8	HO	4	32392	50	0.6962	460	79	370
8	HO	5	32382	65	0.6962	1400	230	700
8	HO	6	32382	80	0.6962	230	33	240
8	HO	C	32382	NM	NM	490	46	390
9	RH	1	41682	5	0.2123	49000	49000	5300
9	RH	2	41682	20	0.5971	170000	170000	1600
9	RH	3	41682	35	0.5971	220000	220000	1800
9	RH	4	41682	50	0.5971	49000	49000	1500
9	RH	5	41682	65	0.5971	49000	49000	1100
9	RH	6	41682	80	0.5971	49000	49000	1400
9	RH	C	41682	NM	NM	170000	130000	1800
10	BH	1	42182	5	0.4245	330	46	320
10	BH	2	42182	20	0.6792	490	70	900
10	BH	3	42182	35	0.6792	700	33	470
10	BH	4	42182	50	0.6792	330	330	520
10	BH	5	42182	65	0.6792	790	790	660
10	BH	6	42182	80	0.6792	490	330	500
10	BH	C	42182	NM	NM	490	490	540
11	RH	1	42382	5	0.4245	140000	33000	1300
11	RH	2	42382	20	0.8037	70000	4000	410
11	RH	3	42382	35	0.8037	79000	22000	420
11	RH	4	42382	50	0.8037	49000	49000	960
11	RH	5	42382	65	0.8037	130000	22000	980
11	RH	6	42382	80	0.8037	70000	33000	870
11	RH	C	42382		NM	79000	18000	760
12	RH	1	43082	5	0.5971	130000	79000	1200
12	RH	2	43082	20	0.8037	170000	79000	1900
12	RH	3	43082	35	0.8037	140000	49000	6700
12	RH	4	43082	50	0.8037	110000	70000	4100
12	RH	5	43082	65	0.8037	170000	70000	3800
12	RH	6	43082	80	0.8037	130000	33000	5100
12	RH	C	43082	NM	NM	130000	64000	1600
13	HO	1	50482	5	0.2745	1700	170	6200
13	HO	2	50482	20	0.5971	490	33	3600
13	HO	3	50482	35	0.6962	220	79	1400
13	HO	4	50482	50	0.6962	39	8	720
13	HO	5	50482	65	0.6962	49	33	870
13	HO	6	50482	80	0.6962	110	11	940
13	HO	7	50482	95	0.6962	79	2	1900
13	HO	C	50482	NM	NM	330	49	1800
14	HO	1	51182	5	0.2745	130	23	280
14	HO	2	51182	20	0.6962	350	79	470
14	HO	3	51182	35	0.6962	330	130	1100
14	HO	4	51182	50	0.6962	1300	110	800
14	HO	5	51182	65	0.6962	1100	230	470
14	HO	6	51182	80	0.6962	490	110	300
14	HO	7	51182	95	0.6962	170	79	420
14	HO	C	51182	NM	NM	[illegible]	95	180

RUN	STATION	SAMPLE	DATE	TIME,MINS.	FLOW,L/SEC	MPN TC	MPN FC	MPN FS
15	BH	1	51882	5	0.1132	4900	170	4300
15	BH	2	51882	20	0.3453	7900	140	5700
15	BH	3	51882	35	0.4245	1300	1300	8500
15	BH	4	51882	50	0.4245	2800	2800	8100
15	BH	5	51882	65	0.4245	2300	2300	8000
15	BH	6	51882	80	0.4245	4900	4900	5400
15	BH	7	51882	95	0.4245	7900	4900	5100
15	BH	C	51882	NM	NM	3300	3300	7500

EB = EAST BALTIMORE SITE
BH = BOLTON HILL SITE
HO = HOMELAND SITE
RH = RESERVOIR HILL SITE

C = COMPOSITE SAMPLE
NM = NOT MEASURED

TC = TOTAL COLIFORMS
FC = FECAL COLIFORMS
FS = FECAL STREP

APPENDIX B. LEVELS OF BACTERIA, SOURCE SAMPLES.

RUN	STATION	SAMPLE	DATE	CATEGORY	MPN TC	MPN FC	MPNFS
0	EB	1	103181	DF	13000000	450000	36000000
0	EB	2	103181	VG	17000000	200000	54000000
0	EB	3	103181	SL	2200000	320000	4100000
0	EB	4	103181	DF	2800000	320000	220000000
0	EB	5	103181	DF	1700000	1300000	30000000
0	EB	6	103181	DF	79000000	79000000	43000000
0	EB	7	103181	SL	110000	27000	350000
1	EB	1	110681	VG	49000	6100	110000
1	EB	2	110681	RF	700000	130000	39000000
1	EB	3	110681	SL	130000	33000	420000
1	EB	4	110681	RE	13000000	1100000	7600000
2	EB	1	110981	RF	1100000	790000	40000000
2	EB	2	110981	RE	110000	31000	40000
2	EB	3	110981	SL	33000	<200	35000
2	EB	4	110981	DF	330000	230000	20000000
2	EB	5	110991	RF	4900000	2200000	34000000
2	EB	6	110991	VG	49000	49000	33000
3	BH	1	111281	SL	4500	<200	1000
3	BH	1	111281	SL	1700	<200	750
3	BH	3	111281	VG	200	<200	13000
3	BH	4	111281	VG	3400	<200	9000
3	BH	5	111281	DF	7800	4500	200000
4	RH	1	112491	DF	49000	49000	7300000
4	RH	2	112481	DF	1300000	33000	130000000
4	RH	3	112481	SL	180	<20	600
4	RH	4	112491	VG	78	<20	2100
4	RH	5	112491	RE	2	<20	1400
4	RH	6	112481	RF	950000	330000	13000000
5	BH	1	120181	DF	79000	23000	360000
5	BH	2	120181	SL	2200	<200	540
5	BH	3	120181	VG	3200	<200	5700
5	BH	4	120181	SL	1300	<200	450
5	BH	5	120191	SL	330	<200	350
6	HO	1	121181	DF	7900000	1400000	9700000
6	HO	2	121181	DF	33000000	23000000	5500000
6	HO	3	121191	SL	130	17	160
6	HO	4	121191	VG	1700	<20	3000
6	HO	5	121181	SL	490	45	<10
6	HO	6	121181	VG	1300	23	430
6	HO	7	121191	DD	1300	5	3000
7	BH	1	22682	DE	4900	3900	27000
7	BH	2	22682	DF	11000	11000	890000
7	BH	3	22682	DF	79000	79000	840000
7	BH	4	22682	DF	790	110	380000
7	BH	5	22682	SL	49	<2	5000
7	BH	6	22682	SL	350	2	1700
7	BH	7	22682	VG	1900	<2	71000
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

RUN	STATION	SAMPLE	DATE	CATEGORY	MPN TC	MPN FC	MPN FS
8	HO	1	32382	DF	130000000	130000000	2900000000
8	HO	2	32382	DF	64000	33000	12000000
8	HO	3	32382	SL	1700	<20	35
8	HO	4	32382	SL	<20	<20	15
8	HO	5	32382	SL	130	8	<20
8	HO	6	32382	DD	83	<20	130
9	RH	1	41682	DF	7900000	7900000	630000000
9	RH	2	41682	DF	1300000000	1300000000	650000000
9	RH	3	41682	DF	13000000	13000000	7800000
9	RH	4	41682	VG	780	<200	5400
9	RH	5	41682	SL	450	<200	11000
9	RH	6	41682	RE	11000	610	92000
9	RH	7	41682	DU	460	130	1600
10	BH	1	42182	DF	13000000	13000000	34000000
10	BH	2	42182	DF	330000	170000	25000000
10	BH	3	42182	DF	1100000	1100000	20000000
10	BH	4	42182	DF	230000	230000	1500000
10	BH	5	42182	VG	110	<20	75
10	BH	6	42182	SL	3300	<200	620
10	BH	7	42182	DD	17000	<200	630
11	RH	1	42382	DF	31000	31000	310000000
11	RH	2	42382	DF	4900000	1700000	230000000
11	RH	3	42382	DF	70000000	70000000	2700000000
11	RH	4	42382	DF	23000	23000	5900000
11	RH	5	42382	SL	7900	72	86000
11	RH	6	42382	RE	<20	<20	2500
11	RH	7	42382	DD	79000	7900	10000
12	RH	1	43082	DF	33000000	33000000	75000000
12	RH	2	43082	DF	2300000	2300000	1400000000
12	RH	3	43082	DF	33000	33000	720000000
12	RH	4	43082	RE	27	<2	1200
12	RH	5	43082	VG	130	33	77000
12	RH	6	43082	SL	33	33	650
12	RH	7	43082	DU	13000	140	13000
13	HO	1	50482	DF	180000000	450000	2300000
13	HO	2	50482	DU	70	<2	1600
13	HO	3	50482	SL	2	<2	340
13	HO	4	50482	SL	<2	<2	10
13	HO	5	50482	VG	79	17	570
13	HO	6	50482	VG	2	<2	5
13	HO	7	50482	SL	23	2	100
14	HO	1	51182	DF	79000000	20000	880000
14	HO	2	51182	DF	700000	460000	80000000
14	HO	3	51182	SL	49	2	110
14	HO	4	51182	SL	5	<2	10
14	HO	5	51182	VG	<2	<2	5
14	HO	6	51182	VG	4	<2	30
14	HO	7	51182	DU	330	110	180

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RUN	STATION	SAMPLE	DATE	CATEGORY	MPN TC	MPN FC	MPN FS
15	BH	1	51882	DF	230000000	230000000	210000000
15	BH	2	51882	DF	79000000	79000000	29000000
15	BH	3	51882	DF	79000000	79000000	110000000
15	BH	4	51882	DF	230000000	230000000	33000000
15	BH	5	51882	VG	7	<2	10
15	BH	6	51882	SL	13	5	2000
15	BH	7	51882	SL	110	11	1800
16	BH	1	61882	VG	1300	13	4200
16	BH	2	61882	VG	8	<2	10
16	BH	3	61882	VG	5	<2	40
16	BH	4	61882	VG	13000	790	35000
16	BH	5	61892	SL	720	79	770
17	RH	1	61882	VG	1100	280	2900
17	RH	2	61882	VG	790	23	6000
17	RH	3	61882	VG	230	14	7900
17	RH	4	61882	SL	13000	330	9900
17	RH	5	61882	SL	1800	170	19000
18	HO	1	61882	VG	330	110	400
18	HO	2	61882	VG	700	33	370
18	HO	3	61892	VG	110	1800	2200
18	HO	4	61882	SL	230	11	4500
18	HO	5	61882	SL	130	23	1800

EB = EAST BALTIMORE SITE
BH = BOLTON HILL SITE
HO = HOMELAND SITE
RH = RESERVOIR HILL SITE

DF = DOG FECES
RF = RAT FECES
SL = SOIL
VG = VEGETATION
RE = REFUSE
DD = STREET SURFACE DUST AND DIRT

TC = TOTAL COLIFORMS
FC = FECAL COLIFORMS
FS = FECAL STREP

Appendix C. F AND P VALUES FROM ANOVA OF 15 TRIALS, COMPARING INDICATOR DENSITIES OF DISCRETE AND COMPOSITE RUNOFF SAMPLES

Run No.		Total Coliform	Fecal Coliform	Fecal Strep
1	F	0.20(NSD)	0.01(NSD)	0.00(NSD)
	P	0.6852	0.9194	0.9868
2	F	0.08(NSD)	0.77(NSD)	0.42(NSD)
	P	0.7950	0.4289	0.5524
3	F	0.00(NSD)	0.03(NSD)	0.07(NSD)
	P	0.9546	0.8733	0.7981
4	F	0.34(NSD)	0.47(NSD)	0.31(NSD)
	P	0.5837	0.5256	0.6006
5	F	0.44(NSD)	0.36(NSD)	0.01(NSD)
	P	0.5360	0.5732	0.9274
6	F	5.28(NSD)	0.00(NSD)	0.10(NSD)
	P	0.0700	0.9920	0.7671
7	F	0.10(NSD)	0.10(NSD)	0.00(NSD)
	P	0.7668	0.7689	0.9475
8	F	0.21(NSD)	0.01(NSD)	0.13(NSD)
	P	0.6671	0.9292	0.7376
9	F	1.04(NSD)	0.45(NSD)	0.00(NSD)
	P	0.3545	0.5313	0.9925
10	F	0.00(NSD)	0.77(NSD)	0.00(NSD)
	P	0.9860	0.4197	0.9684
11	F	0.02(NSD)	0.04(NSD)	0.00(NSD)
	P	0.8984	0.8533	0.9877
12	F	0.16(NSD)	0.02(NSD)	1.05(NSD)
	P	0.7038	0.8970	0.3532
13	F	0.24(NSD)	0.25(NSD)	0.01(NSD)
	P	0.6426	0.6350	0.9282
14	F	0.02(NSD)	0.00(NSD)	0.76(NSD)
	P	0.8799	0.9571	0.4157
15	F	0.05(NSD)	0.37(NSD)	0.40(NSD)
	P	0.8367	0.5677	0.5485

NSD no significant difference